

# **Draft Assessment Report (DAR)**

**- public version -**

**Initial risk assessment provided by the rapporteur Member State  
United Kingdom for the existing active substance**

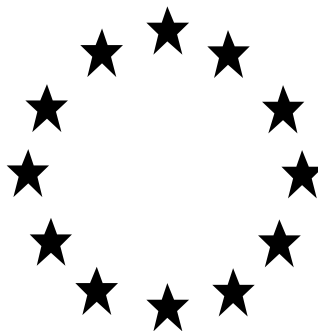
**METHOMYL**

**of the second stage of the review programme referred to in Article 8(2)  
of Council Directive 91/414/EEC**

**Volume 3, Annex B, part 1, B.1 – B.7**

**November 2004**

# **Council Directive 91/414/EEC**



**Methomyl**

**Volume 3**

**Annex B**

**to the Report and Proposed Decision of the United Kingdom  
made to the European Commission under Article 8(1) of  
91/414/EEC**

**Summary, Scientific Evaluation and Assessment**

**Draft: April 2004**

**CONTENTS****Page****B.1 Identity****5**

- B.1.1 Identity of the active substance
- B.1.2 Identity of the plant protection product
- B.1.3 References relied on

**B.2 Physical and chemical properties****8**

- B.2.1 Physical and chemical properties of the active substance
- B.2.2 Physical, chemical and technical properties of the plant protection product
- B.2.3 Summary of physical and chemical properties
- B.2.4 References relied on

**B.3 Data on application and further information****32**

- B.3.1 Data on application relevant to active substance
- B.3.2 Data on application relevant to the plant protection product
- B.3.3 Summary of data on application
- B.3.4 Further information on the active substance
- B.3.5 Further information on the plant protection product
- B.3.6 Summary of further information on the active substance and plant protection product
- B.3.7 References relied on

**B.4 Proposals for classification and labelling****42**

- B.4.1 Proposals for the classification and labelling of the active substance
- B.4.2 Proposals for the classification and labelling of preparations
- B.4.3 References relied on

**B.5 Methods of analysis****43**

- B.5.1 Analytical methods for formulation analysis
- B.5.2 Analytical methods (residue) for treated plants, plant products, foodstuffs of plant and animal origin and feeding stuffs
- B.5.3 Analytical methods (residue) in soil, water and air
- B.5.4 Analytical methods (residue) in human and animal tissues and fluids
- B.5.5 Evaluation and assessment
- B.5.6 References relied on

**B.6 Toxicology and metabolism****63**

- B.6.1 Absorption, distribution, excretion and metabolism (toxicokinetics)
- B.6.2 Acute toxicity, irritancy and skin sensitisation
- B.6.3 Short-term toxicity
- B.6.4 Genotoxicity
- B.6.5 Long-term toxicity and carcinogenicity
- B.6.6 Reproductive toxicity
- B.6.7 Delayed neurotoxicity
- B.6.8 Further toxicological studies
- B.6.9 Medical data and information

B.6.10	Summary of mammalian toxicology and proposed ADI, AOEL, ARfD and MAC (drinking water limit)	178
B.6.11	Acute toxicity including irritancy and skin sensitisation of the preparations	
B.6.12	Dermal absorption	
B.6.13	Toxicological data on non active substances	
B.6.14	Exposure data	208
B.6.15	References relied on	
<b>B.7</b>	<b>Residue data</b>	<b>238</b>
B.7.1	Metabolism, distribution and expression of residues in plants	
B.7.2	Metabolism, distribution and expression of residues in domestic livestock	
B.7.3	Definition of the residue	
B.7.4	Use pattern	
B.7.5	Identification of critical GAPs	
B.7.6	Residues arising from supervised trials	
B.7.7	Stability of residues prior to analysis	
B.7.8	Effects of industrial processing and/or household processing	
B.7.9	Livestock feeding studies	
B.7.10	Residues in succeeding or rotational crops	
B.7.11	Proposed pre-harvest intervals for envisaged uses, or withholding periods, in the case of post harvest uses	
B.7.12	Community MRLs and MRLs in EU Member States	
B.7.13	Proposed EU MRLs and justification for the acceptability of those MRLs	
B.7.14	Proposed EU Import tolerances and justification for the acceptability of those residues	
B.7.15	Basis for differences, if any, in conclusions reached having regard to established or proposed CAC MRLs	
B.7.16	Estimates of potential and actual dietary exposure through diet and other means	
B.7.17	Summary and evaluation of residue behaviour	
B.7.18	References relied on	
B.7.19	References used in assessment of data	
<b>B.8</b>	<b>Environmental fate and behaviour</b>	<b>308</b>
B.8.1	Route and rate of degradation in soil	
B.8.2	Adsorption, desorption and mobility in soil	
B.8.3	Predicted environmental concentrations in soil (PEC <sub>s</sub> )	
B.8.4	Fate and behaviour in water	
B.8.5	Impact on water treatment procedures	
B.8.6	Predicted environmental concentrations in surface water and ground water (PEC <sub>sw</sub> , PEC <sub>sed</sub> , PEC <sub>gw</sub> )	
B.8.7	Fate and behaviour in air	
B.8.8	Predicted environmental concentration in air (PEC <sub>a</sub> )	
B.8.9	Definition of the residue	
B.8.10	References relied on	

<b>B.9</b>	<b>Ecotoxicology</b>	<b>390</b>
B.9.1	Effects on birds	
B.9.2	Effects on aquatic organisms	
B.9.3	Effects on other terrestrial vertebrates	
B.9.4	Effects on bees	
B.9.5	Effects on other arthropod species	
B.9.6	Effects on earthworms	
B.9.7	Effects on other soil non-target macro-organisms	
B.9.8	Effects on soil non-target micro-organisms	
B.9.9	Effects on other non-target organisms (flora and fauna) believed to be at risk	
B.9.10	Effects on biological methods of sewage treatment	
B.9.11	References relied on	
<b>Appendix 1</b>	<b>Standard terms and abbreviations</b>	<b>497</b>
<b>Appendix 2</b>	<b>Specific terms and abbreviations</b>	<b>508</b>
<b>Appendix 3</b>	<b>Material Safety Data Sheets</b>	<b>511</b>
<b>Appendix 4</b>	<b>Operator exposure estimates</b>	<b>516</b>
<b>Appendix 5</b>	<b>Ecotoxicological modelling (birds and mammals)</b>	<b>560</b>
<b>Appendix 6</b>	<b>Metabolites and degradation products</b>	<b>579</b>

WARNING: This document forms part of an EC evaluation data package and should not be read in isolation. Registration must not be granted on the basis of this document.

**B.1 Identity****B.1.1 Identity of the active substance (IIA 1, 3.1)****B.1.1 Name and address of applicant**

**Applicant** DUPONT DE NEMOURS (FRANCE) S.A.S.

**Address** DuPont Crop Protection  
137, rue de l'Université  
F-75334 Paris Cédex 07  
France

**Primary Contact:** [REDACTED]

**Contact and address  
for correspondence:** [REDACTED]

**Telephone:** [REDACTED]

**Telefax:** [REDACTED]

**Email:** [REDACTED]

**B.1.1.2 Common name and synonyms (IIA, 1.3)**

Methomyl (ISO approved), no synonyms

**B.1.1.3 Chemical name (IIA 1.4)**

**CAS Name:** Methyl N-[[[(methylamino)carbonyl]oxy]ethanimidothioate

**IUPAC Name:** S-Methyl N-(methylcarbamoyloxy)thioacetimidate

**B.1.1.4 Manufacturer's development code number (IIA 1.5)**

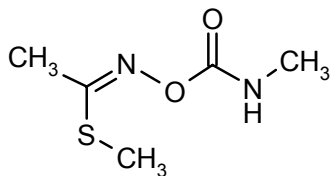
DPX-X1179, IN-X1179

**B.1.1.5 CAS, EEC and CIPAC numbers (IIA 1.6)**

**CAS registry number:** 16752-77-5

**CIPAC number:** 264

**EEC number:** 240-815-0

**B.1.1.6 Molecular and structural formulae, molecular mass (IIA 1.7)****Empirical  
formula****Molecular mass** 162.2**B.1.2 Identity of the plant protection product (IIIA 1)****B.1.2.1 Current, former and proposed trade names and development code numbers (IIIA 1.3)**

Methomyl 20 SL (synonyms: Lannate 20 SL or Methomex)

**B.1.2.2 Type of the preparation and code (IIIA 1.5)**

Soluble concentrate (SL).

**B.1.3 Summary**

Refer to Level 1 of Volume 1 of this Draft Assessment Report

**B.1.3 References relied on**

Author	Annex point	Year	Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or Unpublished	Data protection claimed Y/N	Owner
Schmuckler, M E and Stattmann, M	Annex II Tier II Section 1	Nov 2002	Identity, Physical and chemical properties & Further information Revision 1	Yes	Du Pont

**Plant Protection Product – Methomyl 20 SL**

Author	Annex point	Year	Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or Unpublished	Data protection claimed Y/N	Owner

<b>Author</b>	<b>Annex point</b>	<b>Year</b>	<b>Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or Unpublished</b>	<b>Data protection claimed Y/N</b>	<b>Owner</b>
Cosgrove, T and Stattmann, M	Annex III Tier II Section 1	Nov 2002	Identity, Physical and chemical properties & Further information Revision 1	Yes	Du Pont
Stattmann, M and Ranken, D D .	Document D-1	2001	Methomyl 20% Soluble Concentrate (SL) Details of the intended uses and Conditions of Use (GAP) supported in relation to the proposed inclusion of the plant protection product	Yes	Du Pont

WARNING: This document forms part of an EC evaluation data package and should not be read in isolation. Registration must not be granted on the basis of this document.



**B.2 Physical and chemical properties****B.2.1 Physical and chemical properties of the active substance**

Table B.2.1 Summary of the physical and chemical properties of the active substance (studies were completed to an acceptable standard and results were considered to be valid unless specified otherwise)

section (Annex point)	study	purity	method	results	comment	reference
B.2.1.1 (IIA 2.1)	Melting point	Methomyl (Batch DPX- X1179- 512) (98.02%)	EEC A.1. OECD 102 (capillary method EPA 830.7200)	The melting point of methomyl was measured in triplicate with a digital melting point apparatus and was determined to be $79.6 \pm 0.1^{\circ}\text{C}$ .		Tuffy, C., 2001
B.2.1.2 (IIA 2.1)	Boiling point	Methomyl (DPX- X1179- 512) (98.02%)	EEC A.2. EPA 830.7220	Not applicable; the test material is a solid which decomposes after melting.		Tuffy, C., 2001
B.2.1.3 (IIA 2.1)	Temperature of decomposition or sublimation	Methomyl (DPX- X1179- 512) (98.02%)	EEC A.1.	The decomposition temperature was measured in triplicate and determined to be: $192 \pm 3.1^{\circ}\text{C}$ .	No detailed observations were reported.	Tuffy, C., 2001
B.2.1.4 (IIA 2.2)	Relative density	Methomyl (DPX- X1179- 512) (98.02%)	EEC A.3. OECD 109 EPA 830.7300 Gas pycnometer method	Density of methomyl: $1.318 \pm 0.001 \text{ g/cm}^3$ ( $1318 \text{ kg/m}^3$ ) @ $20.3 \pm 0.4^{\circ}\text{C}$ .	<u>Density</u> , rather than <u>relative density</u> was reported.  <u>Bulk density</u> = 0.57g/ml	Huntley, K., 2001  Silveira E.J. 1989

section (Annex point)	study	purity	method	results	comment	reference
B.2.1.5 (IIA 2.3)	Vapour pressure	Methomyl (DPX- X1179-13- 1-0-0) (>99.2% purity)	EPA 63-9 OTS Guidelines CG1600  Gas saturation method. HPLC used to determine methomyl.	The vapour pressure of methomyl at 25°C was determined to be $5.4 \times 10^{-6}$ mm Hg ( $7.2 \times 10^{-4}$ pascals)	Slightly volatile  Study pre-dates requirement for GLP, but data are acceptable.  HPLC method info and limited validation data. Method accepted.	Barefoot, A.C., Cooke, L.A., 1989
B.2.1.6 (IIA 2.3)	Volatility, Henry's law constant	NA	Calculated	At 25°C:  $2.1 \times 10^{-11}$ atm-m <sup>3</sup> /mole  $2.1 \times 10^{-6}$ pascals-m <sup>3</sup> /mole	Constant indicates low volatalitytic potential from aqueous systems due to high water-solubility.	Barefoot, A.C., Cooke, L.A., 1989
B.2.1.7 (IIA 2.4)	Appearance: physical state	Methomyl (DPX- X1179- 512) (98.02% purity)	None	Solid		Silveira, E.J., 1990
B.2.1.8 (IIA 2.4)	Appearance: colour	Methomyl (DPX- X1179- 512) (98.02% purity)	None	Methomyl is a white powder.		Silveira, E.J., 1990

section (Annex point)	study	purity	method	results	comment	reference
B.2.1.9 (IIA 2.4)	Appearance: odour	Methomyl (DPX- X1179) (92% purity)	None	Slightly sulphurous. The sample was placed approximately 3 inches away from the nose to determine any odour; no strong inhalation was made to avoid personal exposure to dust. The test was conducted at room temperature.		Silveira, E.J., 1990
B.2.1.10 (IIA 2.5)	Spectra					
	NMR	Methomyl (DPX- X1179- 357) (99.8% purity)	None	<p>The assignment of chemical shifts of protons in ppm are given below:</p> <p>2.223 singlet 'vinyl' methyl</p> <p>2.386 singlet S-methyl</p> <p>2.892 doublet N-methyl</p> <p>5.947 broad N-H</p>	<p>Not to GLP, but using Standard Operating Procedures in a GLP facility. Data acceptable.</p> <p>The spectrum is consistent with the structure.</p>	Jeffery, D.J., 2001a
	Mass spectrum	Methomyl (DPX- X1179- 357) (99.8% purity)	<p>None</p> <p>LC-MS (positive ion electrospray)</p>	<p>Assignments:</p> <p>m/z 163 [M+H]<sup>+</sup></p> <p>m/z 106 [HON=C(Me)SMe + H]<sup>+</sup></p> <p>m/z 88 [N=C(Me)SMe]<sup>+</sup></p>	<p>Not to GLP, but using Standard Operating Procedures in a GLP facility. Data acceptable.</p> <p>The spectrum supports the proposed structure for methomyl.</p>	Jeffery, D.J., 2001b

section (Annex point)	study	purity	method	results	comment	reference																
	Infra-red spectrum	Methomyl (DPX- X1179- 357) (99.8% purity))	None  KBr pellet	Absorption bands (cm <sup>-1</sup> ) and assignments:  3301 N-H stretch, carbamate 1509 N-H bend, carbamate 1713 C=O stretch, carbamate 1248 N-C-O stretch, carbamate 1096 C-O stretch 937 =C-S stretch 1599 C=N stretch (variable intensity)	Not to GLP, but using Standard Operating Procedures in a GLP facility. Data acceptable.  Several medium-strong bands.	Jeffery, D.J., 2001c																
	UV / VIS spectrum	Methomyl (DPX- X1179- 357) (99.8% purity)	OECD 101 (1996) OPPTS Series 830.7050 (1996)	UV / VIS absorbance maximum for acidic, basic, and neutral solutions of methomyl was 234 nm(25°C).  <table><thead><tr><th>pH</th><th>λ<sub>max</sub></th><th>ε</th><th>log ε</th></tr></thead><tbody><tr><td>1.74</td><td>234</td><td>8.98 x 10<sup>3</sup></td><td>3.95</td></tr><tr><td>10.92</td><td>234</td><td>8.89 x 10<sup>3</sup></td><td>3.95</td></tr><tr><td>7.02</td><td>234</td><td>9.01 x 10<sup>3</sup></td><td>3.95</td></tr></tbody></table> <ul style="list-style-type: none"><li>No absorption maxima beyond 290nm were observed (all pH conditions). Solutions in methanol at higher concentrations measured over a longer cell path length also showed no absorption maxima beyond 290 nm.</li><li>No effect of pH on absorbance / λ<sub>max</sub> for time periods up to 30 min.</li></ul>	pH	λ <sub>max</sub>	ε	log ε	1.74	234	8.98 x 10 <sup>3</sup>	3.95	10.92	234	8.89 x 10 <sup>3</sup>	3.95	7.02	234	9.01 x 10 <sup>3</sup>	3.95	ε : molar extinction coefficient  λ <sub>max</sub> : absorption maximum  V. low susceptibility to direct photo-degradation at wavelengths ≥ 290nm.	Moore, L.A., 1999
pH	λ <sub>max</sub>	ε	log ε																			
1.74	234	8.98 x 10 <sup>3</sup>	3.95																			
10.92	234	8.89 x 10 <sup>3</sup>	3.95																			
7.02	234	9.01 x 10 <sup>3</sup>	3.95																			

section (Annex point)	study	purity	method	results	comment	reference
B.2.1.10 (IIA 2.5)	Spectra, impurities	-	-	-	The impurity <i>methomyl oxime</i> forms part of the residue definition for food crops, but spectra will not be required. None of the remaining impurities present in the active substance as manufactured are of toxicological or environmental significance	Schmuckler and Stattmann, 2002
B.2.1.11 (IIA 2.6)	Solubility in water	Methomyl (DPX-X1179-362) (96.2% purity)	EPA 63-8 OTS Guidelines CG-1500  Shake flask method  Assay by HPLC	55 mg/mL(25°C)	Not to GLP, but using Standard Operating Procedures in a GLP facility. Data acceptable.  Neutral pH only. Methomyl is non-ionising.  HPLC method info and limited validation data. Method accepted	Hoffman, R.M., 1988

section (Annex point)	study	purity	method	results	comment	reference																				
B.2.1.12 (IIA 2.7)	Solubility in organic solvents (technical active substance)	Methomyl (DPX- X1179- 512) (98.02% purity)	EEC A.6 OECD 105  Shake flask method.  Assay by HPLC	Solubilities at 20°C:  <table><tr><th>Solvent</th><th>Solubility (mg/L)</th></tr><tr><td>Ethyl Acetate</td><td>7.74x10<sup>4</sup></td></tr><tr><td>n-Heptane</td><td>97.1</td></tr><tr><td>1-Octanol</td><td>2.40x10<sup>4</sup></td></tr><tr><td>Xylene</td><td>9.58x10<sup>3</sup></td></tr><tr><td>Acetone</td><td>&gt;250g/kg</td></tr><tr><td>Acetonitrile</td><td>&gt;250g/kg</td></tr><tr><td>Dichloromethane</td><td>&gt;250g/kg</td></tr><tr><td>Dimethylformamide</td><td>&gt;250g/kg</td></tr><tr><td>Methanol</td><td>&gt;250g/kg</td></tr></table>	Solvent	Solubility (mg/L)	Ethyl Acetate	7.74x10 <sup>4</sup>	n-Heptane	97.1	1-Octanol	2.40x10 <sup>4</sup>	Xylene	9.58x10 <sup>3</sup>	Acetone	>250g/kg	Acetonitrile	>250g/kg	Dichloromethane	>250g/kg	Dimethylformamide	>250g/kg	Methanol	>250g/kg	HPLC method info and limited validation data. Method accepted	Moore, L.A., 2001
Solvent	Solubility (mg/L)																									
Ethyl Acetate	7.74x10 <sup>4</sup>																									
n-Heptane	97.1																									
1-Octanol	2.40x10 <sup>4</sup>																									
Xylene	9.58x10 <sup>3</sup>																									
Acetone	>250g/kg																									
Acetonitrile	>250g/kg																									
Dichloromethane	>250g/kg																									
Dimethylformamide	>250g/kg																									
Methanol	>250g/kg																									
B.2.1.13 (IIA 2.8)	Partition co- efficient: n- octanol / water	Methomyl (DPX- X1179 Lot No. CH880) (99.3% purity)	EPA 63-11 OTS Guidelines CG1400  Assay by HPLC	K <sub>ow</sub> of methomyl at 25°C:  = 1.24 (Log K <sub>ow</sub> = 0.09).  Mean of results for two concentrations, differing by a factor 10: K <sub>ow</sub> = 1.14 (at 0.1mg/ml) and 1.35 (at 1mg/ml).  No data for effect of pH (4 – 10). Case made that methomyl does not ionise in environmental pH range.	Not to GLP, but using Standard Operating Procedures in a GLP facility. Data acceptable.  HPLC method info and limited validation data. HPLC method report referenced. Method accepted  Unlikely to bio- accumulate	Singh, H., 1988																				

section (Annex point)	study	purity	method	results	comment	reference
B.2.1.14 (IIA 2.9)	Stability in water				See B.2.1.15 (IIA 2.9)	
B.2.1.15 (IIA 2.9)	Hydrolysis rate	1- <sup>14</sup> C]-methomyl, 95.5% radiochemical purity	EPA Chemical Test Guidelines 560/6-82-003	Hydrolysis of methomyl at 25°C was studied at pH 5, 7, and 9, and at two concentrations, 10 and 100ppm. At pH 9, methomyl hydrolysed with a half-life of ~36 days. Methomyl was stable at pH 5 and 7. The hydrolysis product at pH 9 was IN-X1177 (methomyl-oxime, S-methyl N-hydroxythioacetimidate)	Not to GLP, but study is acceptable. See Section B.8.4.1 Study predates current directive requirement for studies at pH 4. Methomyl degrades by base hydrolysis and is expected to be stable at pH 4. Case accepted.	Friedman, P.L., 1983  Letter response, Aug 2003
B.2.1.16 (IIA 2.9)	Photochemical degradation	[1- <sup>14</sup> C]methomyl (99.0% radiochemical purity)	Analysis by LSC and HPLC / quantification of methomyl by UV. Acceptable information provided.	Methomyl does not undergo direct photolysis. Methomyl does not absorb the energy of sunlight at wavelengths ~290 and above.  When exposed to simulated sunlight, methomyl does undergo indirect photolysis. Methomyl was found to be rapidly degraded by hydroxyl radicals. The degradation rate of [1- <sup>14</sup> C]methomyl (99.0% radiochemical purity) increases with increasing concentrations of nitrate ions in solution. The half-lives of methomyl in summer sunlight equivalent days (39°N) were 45 days (100 molar excess nitrate), 9.5 days (1000 molar excess), and 50 days (pond water). No significant degradation was observed in dark controls, or in irradiated solutions without nitrate.	Study results limited to neutral pH: acceptable as abs max did not change with pH.  Methomyl likely to be rapidly degraded by indirect photolytic process.  See section B.8.4.2	Armbrust, K.L., Reilly, D., 1994

section (Annex point)	study	purity	method	results	comment	reference
B.2.1.17 (IIA 2.9)	Quantum yield	-	-	Not applicable. Methomyl does not absorb the energy of sunlight at wavelengths ~290 and above.	-	Moore, L.A., 1999
B.2.1.18 (IIA 2.9)	Dissociation constant (pKa)	-	-	Not applicable. Methomyl does not ionise over the pH range of environmental concern.	Supported by the pH data for solution in water (pH = 6.6) and consistent with structure of methomyl.	Silveira, E.J., 1990
B.2.1.19 (IIA 2.10)	Stability in air, photochemical oxidative degradation	NA	Calculation (Atkinson method)  OECD and EPA guidelines referenced	Second order rate constant and associated half-life for the reaction of methomyl in the gas phase in the troposphere:  Hydroxyl (OH) rate constant is: $6.6481 \times 10^{-12} \text{ cm}^3 \text{ molecule}^{-1} \text{ sec}^{-1}$ .  The half-life of methomyl with average daily air concentrations of OH radicals (12-hr day; $1.5 \times 10^6$ OH radicals per $\text{cm}^3$ ) is 19.307 hours.	Appropriate guidelines and calculation presented.  Long range transport is unlikely to occur.	Jeffery, D.J., 2001d



section (Annex point)	study	purity	method	results	comment	reference
B.2.1.20 (IIA 2.11)	Flammability and auto-flammability (technical active substance)	EEC A.10. (Flammability)  EEC A 16 (Auto-flammability)	Methomyl (DPX- X1179-512) (98.02% purity) -used for both tests	<p><u>Flammability:</u></p> <p>Methomyl melted but did not ignite, and is classified as not highly flammable. The initial evaluation was performed by applying a gas flame to a small amount of test substance. A full preliminary (train test) was then performed. In the initial test, no ignition occurred and no smoke was observed. On cooling, the residue was clear and colourless with white crystals around the edges. Closely similar observations were made during the train test</p> <p><u>Auto-flammability:</u></p> <p>The temperature/time curve relating to conditions in the center of the sample showed no exotherm of sufficient magnitude to constitute a self-ignition temperature. After heating, a clear, colourless melted residue was present.</p>	<p>Not classified as highly flammable</p> <p>No self-ignition below 110°C</p>	Bates, M., 2001
B.2.1.21 (IIA 2.12)	Flash point (technical active substance)	-	-	-	Not applicable as the active substance is a solid and does not have a melting point below 40°C.	-

section (Annex point)	study	purity	method	results	comment	reference
B.2.1.22 (IIA 2.13)	Explosive properties (technical active substance)	EEC A.14.	Methomyl (DPX- X1179-512) (98.02% purity)	No thermal sensitivity, or mechanical sensitivity to shock or friction was detected.	Not classified as explosive	Bates, M., 2001
B.2.1.23 (IIA 2.15)	Oxidising properties (technical active substance)	-	Reasoned case	Methomyl is clearly non-oxidising, as both of its nitrogen atoms are in the highly reduced -3 oxidation state (Roberts and Caserio, Basic Principles of Organic Chemistry, 1965, p.671).	Case accepted	Schmuckler and Stattmann, 2002
B.2.1.24 (IIA 2.14)	Surface tension	OECD method 15.	Methomyl (DPX- X1179-512) (98.02% purity)	0.0737 N/m (1mg/ml solution in water at 20.1 ± 0.3°C).		Huntley, K., 2001

**B.2.2 Physical, chemical and technical properties of the plant protection product**

Product name: 'Methomyl 20 SL'

Table B.2.2 Summary of the physical and chemical properties of the plant protection product

Test material (unless otherwise stated): Methomyl 20SL (DPX-X1179-524) 204 g/L a.s. (For specification, see Volume 4, Annex C)

section (Annex point)	study	method	results	comment	reference
B.2.2.1 (IIIA 2.1)	Appearance: physical state	Visual assessment	Liquid		Jourdainne, 1999
B.2.2.2 (IIIA 2.1)	Appearance: colour	Visual assessment	Blue liquid		Jourdainne, 1999
B.2.2.3 (IIIA 2.1)	Appearance: odour	Olfactory assessment	This product has a slightly alcoholic odour.		Jourdainne, 1999
B.2.2.4 (IIIA 2.2)	Explosive properties	EEC A.14	No evidence of thermal or mechanical sensitivity under standard test conditions  Mechanical Sensitivity with respect to friction is not required for liquids.		Bates, 1999
B.2.2.5 (IIIA 2.2)	Oxidising properties	EEC A 17	EEC Method A.17 is not applicable to liquids. However, it should be noted that methomyl is clearly non-oxidizing as both nitrogen atoms are in the highly reduced –3 oxidation state (Roberts and Caserio, Basic Principles of Organic Chemistry, 1965, p. 671).	Reasoned case acceptable.	Cosgrove and Stattmann, 2002

section (Annex point)	study	method	results	comment	reference
B.2.2.6 (IIIA 2.3)	Flammability	-	-	See Flash point	-
B.2.2.7 (IIIA 2.3)	Auto-flammability	ASTM-E 659-78 conforming to the requirements of EEC A.15	Auto-ignition temperature = 304 °C at 99.5kPa atmospheric pressure.	Method accepted as equivalent to EEC A.15	Bates, 1999
B.2.2.8 (IIIA 2.3)	Flash point	EEC A.9	The flash point = 34.5°C.	Classified as flammable as flash point is < 55°C	Jourdainne, 1999
B.2.2.9 (IIIA 2.4)	Acidity/alkalinity	-	Declared 'not applicable' in view of result for CIPAC MT 75 (below).	Data gap: directive requires pH of preparation. However formulation composition raises no safety concerns.	Cosgrove and Stattmann, 2002
B.2.2.10 (IIIA 2.4)	pH	CIPAC MT 75	The pH of Methomyl 20SL in a 1% aqueous dilution with distilled water is 4.9.		Jourdainne, 1999
B.2.2.11 (IIIA 2.5)	Surface tension	EEC method A 5	Surface tension: = 37.4 mN/m.		Jourdainne, 1999

section (Annex point)	study	method	results	comment	reference
B.2.2.12 (IIIA 2.5)	Viscosity	-	-	Kinematic viscosity not required, as this preparation was not designed for Ultra Low Volume (ULV) use.  Viscosity measurement not required for a Newtonian liquid.  Case: all components are in solution; liquid is by definition Newtonian. Case accepted	Cosgrove and Stattmann, 2002  Letter response, Aug 2003
B.2.2.13 (IIIA 2.6)	Density	EEC Method A 3	Density of the test substance was 1.036 g/ml at 20°C		Jourdainne, 1999
B.2.2.14 (IIIA 2.6)	Bulk (tap) density	-	-	Bulk density measurements do not apply to liquid preparations.	Cosgrove and Stattmann, 2002

section (Annex point)	study	method	results	comment	reference
B.2.2.14 (IIIA 2.7)	<u>Storage stability</u>  Accelerated storage conditions: 54°C for 2 weeks.          Cold temperature storage (0°C)	CIPAC Method MT 46  An. Method: X1179.220.05. LP  CIPAC MT 75    CIPAC MT 39	<u>Active content.</u> (g/l)  Start		

section (Annex point)	study	method	results	comment	reference
B.2.2.15 (IIIA 2.7)	Shelf life  Storage at ambient temperature for 2 years	GIFAP Monograph No 17  An. Method: X1179.220.06. ES	<u>Appearance</u>  Blue liquid, alcoholic odour. No change after storage with no evidence of phase separation.  <u>Active cont. (g/l)</u>  Start                      End 204                      210  <u>pH</u>  Start                      End 4.9                      4.4  <u>Container</u>  There was no evidence of any seepage or leakage from the (HDPE) container nor was there any indication of panelling (change of shape).	Analytical method acceptably validated.	Bloemer, 2001
B.2.2.16 (IIIA 2.8)	Wettability	-	-	Not applicable	-
B.2.2.17 (IIIA 2.8)	Persistent foaming	CIPAC Method MT 47	No foam after standing undisturbed for 1 minute. (Using CIPAC standard hard water "D" and highest use rate: 0.45ml/100ml water).	Acceptable result for higher use rate extrapolated to lower use rate.	Jourdainne, 1999

section (Annex point)	study	method	results	comment	reference
B.2.2.18 (IIIA 2.8)	Suspensibility	-	-	Not applicable	-
B.2.2.19 (IIIA 2.8)	Suspension stability	-	-	Not applicable	-
B.2.2.20 (IIIA 2.8)	Dilution stability	CIPAC Method MT 41	A 0.45-gram sample diluted to 100 mL with CIPAC Standard Water "D" and allowed to stand undisturbed for a period of 18 hours at 20°C:  No evidence of any separation of material.	-	Jourdainne, 1999
B.2.2.21 (IIIA 2.8)	Dry sieve test	-	-	Not applicable	-
B.2.2.22 (IIIA 2.8)	Wet sieve test	-	-	Not applicable	-
B.2.2.23 (IIIA 2.8)	Particle size distribution	-	-	Not applicable	-
B.2.2.24 (IIIA 2.8)	Content of dust/fines	-	-	Not applicable	-
B.2.2.25 (IIIA 2.8)	Attrition and friability	-	-	Not applicable	-



<b>section (Annex point)</b>	<b>study</b>	<b>method</b>	<b>results</b>	<b>comment</b>	<b>reference</b>
B.2.2.26 (IIIA 2.8)	Emulsifiability, re-emulsifiability and emulsion stability	-	-	Not applicable	-
B.2.2.27 (IIIA 2.8)	Stability of dilute emulsion	-	-	Not applicable	-
B.2.2.28 (IIIA 2.8)	Flowability	-	-	Not applicable	-
B.2.2.29 (IIIA 2.8)	Pourability (rinsibility)	-	-	Not applicable	-
B.2.2.30 (IIIA 2.8)	Dustability	-	-	Not applicable	-
B.2.2.31 (IIIA 2.8)	Adherence and distribution to seeds	-	-	Not applicable	-

**B.2.2.32 Summary of physical and chemical compatibility with other products (IIIA 2.9)**

For methomyl, no use with other products, including plant protection products, needs to be authorised in the context of which this dossier is submitted.

See physical compatibility (§2.9.1) of Document 1663/VI/94 Rev. 8 of 22-Apr-1998.

**B.2.3 Summary of physical and chemical properties****B.2.3.1 Active substance**

The active substance, methomyl, is a white powder with a slightly sulphurous odour. Methomyl melts at  $79.6 \pm 0.1^\circ\text{C}$  and decomposes at  $192 \pm 3.1^\circ\text{C}$ . The vapour pressure ( $7.2 \times 10^{-4}$  pascals at  $25^\circ\text{C}$ ) indicates that methomyl is slightly volatile but the Henry's law constant ( $2.1 \times 10^{-6}$  pascals/mole) indicates volatilisation from aqueous systems should be low due to its high water solubility. Methomyl should not bio-accumulate since methomyl is soluble in water (55 mg/mL) and the Kow is low (1.24). The log Kow is 0.09. Methomyl does not ionise at the environmentally relevant pH range and is stable in water. Methomyl does not absorb the energy in sunlight at wavelengths of 290 nm and above and no direct photolysis occurs, although indirect photolysis has been observed in the presence of nitrate. Methomyl is soluble in all representative organic solvents. Methomyl is expected to dissipate from the troposphere (~ 19 hours) through reaction with hydroxyl radicals. Methomyl is not flammable, and has no explosive properties, and is non-oxidising, thus, methomyl should present no hazard in shipment or storage.

**Data gaps:** none

**B.2.3.2 Plant protection product**

Methomyl 20SL Soluble Concentrate has a flash point of  $34.5^\circ\text{C}$  and is therefore classified as flammable. It has an auto ignition temperature of  $304^\circ\text{C}$ . It is not explosive nor is it an oxidiser. The pH of a 1% concentration of the preparation in water was measured at 4.9 pH units, both before and after accelerated storage. The active ingredient content of Methomyl 20SL as manufactured was 204 g/L. The physical and chemical properties of the preparation were measured using CIPAC, ASTM and EEC Methods. All physical and chemical property specifications, as defined by "The Manual on the Development and Use of FAO Specifications for Plant Protection Products," were met both prior to and after completion of accelerated storage. The preparation was stored at a temperature of  $54^\circ\text{C}$  for a period of 2 weeks. Acceptable stability was also demonstrated under ambient warehouse conditions after two years' storage.

**B.2.4 References relied on**

Author	Annex point	Year	Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or Unpublished	Data protection claimed Y/N	Owner
Armbrust, K.L., Reilly, D.	IIA, 2.9.2./01	1995	Indirect photodegradation of methomyl in aqueous solutions DuPont Experimental Station AMR 2975-94 GLP: Yes Published: No	Y	DuPont
Barefoot, A.C., Cooke, L.A.	IIA, 2.3.1./01	1989	Vapor pressure of methomyl DuPont Experimental Station AMR 1268-88 GLP: No Published: No	Y	DuPont
Barefoot, A.C., Cooke, L.A.	IIA, 2.3.2./01	1989	Vapor pressure of methomyl DuPont Experimental Station AMR 1268-88 GLP: No Published: No	Y	DuPont
Bates, M.	IIA, 2.11.1./01	2001	Methomyl technical: flammability, relative self-ignition and explosive properties Covance Laboratories, Ltd., SafePharm Laboratories, Ltd. DuPont-5477 GLP: Yes Published: No	Y	DuPont
Bates, M.	IIA, 2.11.2./01	2001	Methomyl technical: flammability, relative self-ignition and explosive properties Covance Laboratories, Ltd., SafePharm Laboratories, Ltd. DuPont-5477 GLP: Yes Published: No	Y	DuPont
Bates, M.	IIA, 2.13./01	2001	Methomyl technical: flammability, relative self-ignition and explosive properties Covance Laboratories, Ltd., SafePharm Laboratories, Ltd. DuPont-5477 GLP: Yes Published: No	Y	DuPont
Friedman, P.L.	IIA, 2.9.1./01	1983	Hydrolysis of [1-14C]methomyl DuPont Experimental Station AMR 109-83 GLP: No Published: No	Y	DuPont
Hoffman, R.M.	IIA, 2.6./01	1988	Determination of the water solubility of methomyl - X1179 DuPont Experimental Station X1179.B GLP: No Published: No	Y	DuPont

Author	Annex point	Year	Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or Unpublished	Data protection claimed Y/N	Owner
Huntley, K.	IIA, 2.2./01	2001	The relative density of methomyl ABC Laboratories, Inc. DuPont-6278 GLP: Yes Published: No	Y	DuPont
Huntley, K.	IIA, 2.14./01	2001	The surface tension of methomyl ABC Laboratories, Inc. DuPont-6277 GLP: Yes Published: No	Y	DuPont
Jeffery, D.J.	IIA, 2.5.1./01	2001a	Methomyl (DPX-X1179): [1]H-NMR spectrum DuPont Stine-Haskell Research Center DuPont-4569 GLP: Yes Published: No	Y	DuPont
Jeffery, D.J.	IIA, 2.5.1./02	2001b	Methomyl (DPX-X1179): mass spectrum (MS) DuPont Stine-Haskell Research Center DuPont-4568 GLP: Yes Published: No	Y	DuPont
Jeffery, D.J.	IIA, 2.5.1./03	2001d	Methomyl (DPX-X1179): IR spectrum DuPont Stine-Haskell Research Center DuPont-4570 GLP: Yes Published: No	Y	DuPont
Jeffery, D.J.	IIA, 2.10./01	2001c	The stability in air of methomyl (DPX-X1179): Atkinson calculation DuPont Stine-Haskell Research Center DuPont-4653 GLP: Yes Published: No	Y	DuPont
Moore, L.A.	IIA, 2.5.1./04	1999	UV/visible absorption of methomyl DuPont Experimental Station DuPont-3116 GLP: Yes Published: No	Y	DuPont
Moore, L.A.	IIA, 2.7./01	2001	Solubility of methomyl (DPX-X1179) in organic solvents DuPont Stine-Haskell Research Center DuPont-4566 GLP: No Published: No	Y	DuPont

Author	Annex point	Year	Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or Unpublished	Data protection claimed Y/N	Owner
Moore, L.A.	IIA, 2.9.3./01	1999	UV/visible absorption of methomyl DuPont Experimental Station DuPont-3116 GLP: Yes Published: No	Y	DuPont
Schmuckler, M E and Stattmann, M	Annex II Tier II Section 1	Nov 2002	Identity, Physical and chemical properties & Further information Revision 1	Yes	Du Pont
Silveira, E.J.	IIA, 2.4.1./01	1990	Technical methomyl - physical and chemical characteristics DuPont Experimental Station AMR 1753-90 GLP: No Published: No	Y	DuPont
Silveira, E.J.	IIA, 2.4.2./01	1990	Technical methomyl - physical and chemical characteristics DuPont Experimental Station AMR 1753-90 GLP: No Published: No	Y	DuPont
Silveira, E.J.	IIA, 2.9.4./01	1990	Technical methomyl - physical and chemical characteristics DuPont Experimental Station AMR 1753-90 GLP: No Published: No	Y	DuPont
Singh, H.	IIA, 2.8./01	1988	N-octanol/water partition coefficient determination of methomyl in distilled water Enviro-Bio-Tech, Ltd. AMR 1234-88 GLP: No Published: No	Y	DuPont
Tuffy, C.	IIA, 2.1.1./01	2001	The melting point/thermal decomposition of methomyl ABC Laboratories, Inc. DuPont-6279 GLP: Yes Published: No	Y	DuPont
Tuffy, C.	IIA, 2.1.2./01	2001	The melting point/thermal decomposition of methomyl ABC Laboratories, Inc. DuPont-6279 GLP: Yes Published: No	Y	DuPont
Tuffy, C.	IIA, 2.1.3./01	2001	The melting point/thermal decomposition of methomyl ABC Laboratories, Inc. DuPont-6279 GLP: Yes Published: No	Y	DuPont

**Plant Protection Product – ‘Methomyl 20 SL’**

<b>Author</b>	<b>Annex point</b>	<b>Year</b>	<b>Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or Unpublished</b>	<b>Data protection claimed Y/N</b>	<b>Owner</b>
Bates, M.	IIIA, 2.2.1./01	1999	Lannate □ 20L: determination of physico-chemical safety properties Covance Laboratories, Ltd., SafePharm Laboratories, Ltd. 550/70-D2141 GLP: Yes Published: No	N	DuPont
Bates, M.	IIIA, 2.3./02	1999	Lannate □ 20L: determination of physico-chemical safety properties Covance Laboratories, Ltd., SafePharm Laboratories, Ltd. 550/70-D2141 GLP: Yes Published: No	N	DuPont
Bloemer, D.S.	IIIA, 2.7.3./01	2001	Shelf life stability of methomyl 200 g/liter (DPX-X1179) soluble concentrate insecticide formulation DuPont Stine-Haskell Research Center DuPont-3093 GLP: No Published: No	N	DuPont
Cosgrove, T and Stattmann, M	Annex III Tier II Section 1	Nov 2002	Identity, Physical and chemical properties & Further information Revision 1	Yes	Du Pont
Jourdainne, C.	IIIA, 2.1./01	1999	Physical, chemical and technical properties of DPX-X1179 (methomyl) 200 g/liter soluble concentrate insecticide formulation DuPont de Nemours (France) S.A. DuPont-3094 GLP: Yes Published: No	N	DuPont
Jourdainne, C.	IIIA, 2.3./01	1999	Physical, chemical and technical properties of DPX-X1179 (methomyl) 200 g/liter soluble concentrate insecticide formulation DuPont de Nemours (France) S.A. DuPont-3094 GLP: Yes Published: No	N	DuPont

Author	Annex point	Year	Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or Unpublished	Data protection claimed Y/N	Owner
Jourdainne, C.	IIIA, 2.4.2./01	1999	Physical, chemical and technical properties of DPX-X1179 (methomyl) 200 g/liter soluble concentrate insecticide formulation DuPont de Nemours (France) S.A. DuPont-3094 GLP: Yes Published: No	N	DuPont
Jourdainne, C.	IIIA, 2.5.3./01	1999	Physical, chemical, and technical properties of DPX-X1179 (methomyl) 200 g/liter soluble concentrate insecticide formulation DuPont de Nemours (France) S.A. DuPont-3094 GLP: Yes Published: No	N	DuPont
Jourdainne, C.	IIIA, 2.6.1./01	1999	Physical, chemical and technical properties of DPX-X1179 (methomyl) 200 g/liter soluble concentrate insecticide formulation DuPont de Nemours (France) S.A. DuPont-3094 GLP: Yes Published: No	N	DuPont
Jourdainne, C.	IIIA, 2.7.1./01	1999	Physical, chemical and technical properties of DPX-X1179 (Methomyl) 200 g/liter soluble concentrate insecticide formulation DuPont de Nemours (France) S.A. DuPont-3094 GLP: Yes Published: No	N	DuPont
Jourdainne, C.	IIIA, 2.7.2./01	1999	Physical, chemical and technical properties of DPX-X1179 (methomyl) 200 g/liter soluble concentrate insecticide formulation DuPont de Nemours (France) S.A. DuPont-3094 GLP: Yes Published: No	N	DuPont
Jourdainne, C.	IIIA, 2.8.2./01	1999	Physical, chemical and technical properties of DPX-X1179 (methomyl) 200 g/liter soluble concentrate insecticide formulation DuPont de Nemours (France) S.A. DuPont-3094 GLP: Yes Published: No	N	DuPont

<b>Author</b>	<b>Annex point</b>	<b>Year</b>	<b>Title</b> <b>Source (where different from company)</b> <b>Company, Report No.</b> <b>GLP or GEP status (where relevant)</b> <b>Published or Unpublished</b>	<b>Data protection claimed</b> <b>Y/N</b>	<b>Owner</b>
Jourdainne, C.	IIIA, 2.8.4./01	1999	Physical, chemical and technical properties of DPX-X1179 (methomyl) 200 g/liter soluble concentrate insecticide formulation DuPont de Nemours (France) S.A. DuPont-3094 GLP: Yes                      Published: No	N	DuPont

WARNING: This document forms part of an EC evaluation data package and should not be read in isolation. Registration must not be granted on the basis of this document.



**B.3 Data on application and further information****B 3.1 Data on application relevant to active substance (IIA 3.1 to 3.6)****B 3.1.1 Function (IIA 3.1)**

Agricultural insecticide/acaricide.

**B.3.1.2 Effects on harmful organisms (IIA 3.2)**

The principal notifier states, “*Methomyl belongs to the chemical class of carbamate pesticides. It exerts its control on harmful organisms by a neurotoxic mechanism. Toxic effects are fairly rapid, leading to paralysis and death of arthropods at normal use rates.*

*Methomyl is neurotoxic and affects the normal functioning of the central nervous system of the pest species. Nervous system functioning is disrupted by the action of methomyl on the acetylcholinesterase system at the synapse of the nerve axons. Inhibition of the enzyme acetylcholinesterase by methomyl results in the blockage of nerve signals, resulting in paralysis and death. Entry of methomyl to the target site is through the cuticle (contact) or by ingestion.*

*Methomyl is not systemic in plants; however, limited translaminar activity has been observed”.*

**B.3.1.3 Field of use (IIA 3.3)**

Field agriculture and vineyards

**B.3.1.4 Harmful organisms controlled (IIA 3.3)**

A variety of biting, chewing, and sucking insect pests of various crops. The notifier asserts that methomyl is active as an ovicide, larvicide, and adulticide on a number of important species, such as *Helicoverpa* and *Spodoptera*.

**B 3.1.5 Mode of action (IIA 3.5)**

According to the notifier, “*Methomyl inhibits the acetylcholinesterase enzyme in insect synapses. The conductance of the nerve impulse, which requires acetylcholinesterase functioning from pre- to post-synapse, is thus disrupted.*

*All data of research and experimentation on the biochemical activity of carbamates in general and methomyl, in particular, suggest that this is the predominant mode of action.*

*Methomyl poisoning of the insect central nervous system causes competitive inhibition of the transmitter system of the synaptic acetylcholinesterase complex. Disruption of this system results in death of the target harmful insect organism. This activity occurs when insects feed on material treated with methomyl and maximum effect from both direct and contact action is evident two days after treatment. Methomyl is an anti-*

*acetylcholine-esterase material and inhibits the functionality of the nerve. In order to pass the nerve impulse across the synapse, a neuro-transmitting substance must be produced. The pre-synaptic nerve produces acetylcholine. Acetylcholine passes across the synapse and binds to the acetylcholine receptor on the post synaptic nerve thereby transmitting the impulse. In order to restore the nerve to the original status, the acetylcholine is broken down by an enzyme acetylcholine-esterase. Methomyl binds to this enzyme preventing the breakdown of the acetylcholine. Thus, the nerve impulse continues producing tremors and excitation of the insect culminating in the death of the insect.”*

**B 3.1.6 Information on the occurrence or possible occurrence of the development of resistance and appropriate management strategies (IIA 3.6)**

The notifier has stated, *“Because the development of resistance cannot be predicted, the use of methomyl should conform to resistance management strategies established for the region.*

*Some insects are known to develop resistance to products used repeatedly for control. When this occurs, the recommended dosages fail to suppress the pest population below the economic threshold. The development of resistance cannot be predicted and local advisors should be consulted for detailed recommendations. These strategies may include incorporation of cultural and biological control practices, alteration of active classes of insecticides on succeeding generations, and targeting the most susceptible life stage.*

*In general the following points will help to avoid resistance.*

- a. Alternate compounds of different chemical classes. Methomyl is a carbamate and can be effectively alternated with pyrethroids and organophosphates.*
- b. When tank mixing, ensure that compounds mixed are not all of the same chemical class.*
- c. Monitor the insect populations and apply methomyl when locally determined economic thresholds are reached.*
- d. Follow label recommendations for rates and spray intervals. Because of methomyl's mode of action and short persistence (thus reducing selection pressure), methomyl has been found to be a valuable tool in resistance management programs”*

**B 3.2 Data on application relevant to the plant protection product – ‘Methomyl 20 SL’ (IIIA 3.1 to 3.9)**

Methomyl is an ovicide, larvicide and adulticide on insect pest species in grapes, courgette, cucumber, tomato and aubergine. Refer to Level 1, Volume one.

**B.3.3 Summary of data on application****B.3.4 Further information on the active substance (IIA 3.7 to 3.9)****B.3.4.1 Recommended methods and precautions concerning handling, storage, transport and fire (IIA 3.7)**

The notifier has proposed the following:

- Handling:** *Technical measures/Precautions: Provide appropriate exhaust ventilation at places where dust is formed. Use only in area provided with appropriate exhaust ventilation.*  
*Safe handling advice: Use personal protective equipment. In case of insufficient ventilation, wear suitable respiratory equipment. Keep away from heat and sources of ignition. Do not breathe dust. Avoid contact with skin and eyes.*
- Storage:** *Technical measures/Storage conditions: Keep container tightly closed in a dry, cool and well-ventilated place. Keep locked-up. Store in a place accessible by authorised persons only.*
- Transport:** ADR/RID  
*Class: 6.1; Item: 73(b); TREM-CARD: 61G41b-A*  
*Packaging group: II; HI-No. 60; SI-No.: 2757*  
*Proper shipping name: 2757 Carbamate pesticide, solid, toxic (methomyl 98%), 6.1, 73(b), ADR*
- IMO  
*Class: 6.1; UN-No.: 2757; Packaging group: II*  
*IMDG Page: 2/132; EmS: 6.1-01; MFAG: Cat. A,*  
*Hazard labels: 6.1, Marine pollutant mark*  
*Proper shipping name: Carbamate pesticide, solid, toxic (methomyl 98%), Class 6.1, UN 2757, PG II, Marine Pollutant*
- ICAO  
*Class: 6.1; UN/ID No.: 2757; Packaging group: II*  
*Hazard labels: 6.1*  
*Packaging instruction (passenger aircraft): 613/25 kg (forbidden)*  
*Packaging instructions (cargo aircraft): 615/200 kg (forbidden)*  
*Proper shipping name: Carbamate pesticide, solid, toxic (methomyl), Class 6.1, UN 2757, PG II*  
*Further information: According to an internal company decision, DuPont does not allow this product to be transported by air.*
- Fire** *Special protective equipment for firefighters: Wear self-contained breathing apparatus and protective suit.*  
*Specific methods: If area is heavily exposed to fire, and, if conditions permit, let fire burn itself out since water may increase the area contaminated. Cool containers/tanks with spray water.*
- Extinguishing media:** *Suitable extinguishing media: dry chemical, water spray.*  
*Extinguishing media which must not be used for safety reasons: high volume water jet (contamination risk).*

**Combustion gases:** *In the event of fire, the formation of hydrogen cyanide, sulphur dioxide, and methyl isocyanate must be anticipated. Further information: Fire or intense heat may cause violent rupture of packages. During processing, dust may form explosive mixtures in air.*

#### B.3.4.2 Procedures for destruction or decontamination (IIA 3.8.1, IIA 3.8.2)

The notifier has stated that a specific study on thermal decomposition has not been carried out. Current practice is to incinerate at a temperature greater than 900°C with a residence time of 2-4 sec in the chamber. Oxygen supply should be adjusted to generate <100 ppm CO in the stack. The notifier states that consideration of content of halogens is not relevant (the molecule is halogen-free).

With respect to the disposal of waste packaging, the applicant proposes, “*Close and label the waste receptacles and, likewise, any uncleaned containers. Dispose of them at a suitable waste incineration plant and/or in accordance with local and national regulations. They must be incinerated in a suitable incineration plant holding a permit delivered by the competent authorities. Where large quantities are concerned, consult the supplier*”.

Dossier references to cleaning procedures were provided via letter (August 2003). Procedures are acceptable and were supplemented by a further report on the effectiveness of cleaning procedures (October 2003). This report demonstrated that the procedures specified are effective and was acceptable (

Huby, J.P., Marquet, F., DuPont-13768 EU 2003).

#### B.3.4.3 Emergency measures in case of an accident (IIA 3.8.2, IIA 3.9)

The notifier has proposed the following:

**Personal precautions:** *Wear self-contained breathing apparatus and protective suit. Evacuate personnel to safe areas.*

**Environmental precautions:** *Do not flush into surface water or sanitary sewer system. This material and its container must be disposed of as hazardous waste.*

**Methods for cleaning up:** *Neutralise with sodium hydroxide and allow to stand for 4 hours. Use approved industrial vacuum cleaner for removal. Shovel into suitable container for disposal.*

#### B.3.5 Further information on the plant protection product (IIIA 4)

##### B.3.5.1 Packaging (type, materials, size etc.), compatibility of the preparation with the proposed packaging materials (IIIA 4.1)

Product name: Methomyl 20 SL

The notifier states that Methomyl 20SL will be packed in 1L, 5L, and 10L bottles:

<b>1L pack size:</b>	10 bottles are grouped into a corrugated box.
Material:	High density polyethylene (HDPE)
Shape/size:	Rectangular based bottle
	Approximated size: 141 (L) x 90 (W) x 163 (H) mm
Opening:	63 mm diameter
Closure:	Screw cap with sealing disk
<b>5L pack size:</b>	4 bottles are grouped into a corrugated box.
Material:	High density polyethylene (HDPE)
Shape/size:	Rectangular based bottle
	Approximated size: 183 (L) x 151 (W) x 283 (H) mm
Opening:	63 mm diameter
Closure:	Screw cap with sealing disk
<b>10L pack size:</b>	
Material:	High density polyethylene (HDPE)
Shape/size:	Rectangular based bottle
	Approximated size: 244 (L) x 195 (W) x 330 (H) mm
Opening:	DIN 60 diameter
Closure:	Screw cap

#### B.3.5.2 Procedures for cleaning application equipment (IIIA 4.2)

The notifier has proposed the following:

##### ***a. Procedures for cleaning application equipment***

*Application equipment should be rinsed thoroughly with water. Clean all spray equipment immediately following application. Drain spray equipment. Thoroughly rinse sprayer and flush the hoses, boom, and nozzles with clean water. Loosen and physically remove visible deposits. Remove and clean nozzles, screens, strainers. Flush the entire system with clean water. Always wear protective clothing when cleaning equipment. Do not clean near wells, water sources, or near desirable vegetation. Dispose of waste rinse water in adequate places.*

##### ***b. Procedures for cleaning protective clothing***

*Wash all protective clothing thoroughly after use.*

##### ***c. Effectiveness of the cleaning procedures***

WARNING: This document forms part of a package and should not be used in isolation. Registration must not be granted on the basis of this document.

*The tank cleanout procedure can be regarded as effective due to the complete water-solubility of Methomyl 20SL as shown under data point 2.0 in this document.*

### **B.3.5.3 Re-entry periods, necessary waiting periods or other precautions to protect man, livestock and the environment (IIIA 4.3)**

The notifier has proposed the following:

<b>Crops</b>	<b>Proposed pre-harvest intervals</b>
<i>Grape (table &amp; wine)</i>	<i>14 days</i>
<i>Tomato/eggplant</i>	<i>7 days</i>
<i>Cucumber/courgette</i>	<i>30 days</i>

**Re-entry period for livestock in grazing areas:** Vineyards, fruiting vegetable plots, and vegetable plots are not intended for cattle grazing. Exposure of livestock though grazing is highly unlikely. As a result, the establishment of a re-entry period for livestock is not necessary.

**Re-entry period for humans to crops, buildings or spaces treated:** Re-entry exposure is predominantly via the dermal route. The inhalation exposure is only important during a relatively short period after application. The activities before harvest (e.g., thinning and pruning) are more intense in grapes as compared to vegetables. Thus, data from the dislodgeable residue decline study in grapes, summarised in data point 6.3.6 of Document M-II (DuPont-5885), were taken to estimate worker exposure to methomyl. The exposure to methomyl is estimated to be 0.026-mg/kg bw/day, which is 5.2% of the dermal AOEL of 0.9-mg/kg bw/day. The re-entry period of 48 hours for grapes and the remaining crops is, therefore, fully justified and safe for the workers.

**Withholding period for animal feeding stuffs:** Grapes and tomatoes are not intended for cattle grazing and exposure of livestock this way is highly unlikely. In addition, residues of the crop or processing are no longer given as foodstuff to domestic animals. For these reasons, the establishment of a withholding period to protect livestock is not considered necessary.

**Waiting period between application and handling treated products:** Not applicable since grapes and vegetables are not collected/handled before harvest.

**Waiting period between application and sowing or planting of succeeding crops:** Grapes is not a crop for which rotational crops are relevant. Moreover, for vegetables and all crop types, a waiting period between last application and sowing or planting succeeding crops is not necessary given the short residual nature of methomyl in soil. Rate of degradation studies confirm the  $DT_{50}$  of methomyl to be 4-8 days. The irrelevance of a waiting period is consistent with the general scenario recognised wherein any study of rotational crops is unnecessary for less than 10% of residual applied active substance and bio-available metabolites being detected after 100 days in soil. No significant residues of methomyl or metabolites, which could lead to residues above the limit of determination at harvest, are expected to remain in soil or plant materials up to sowing or planting time of succeeding crops.

#### B.3.5.4 Recommended methods and precautions concerning handling, storage, transport or fire (IIIA 4.4)

The notifier has proposed the following:

**Handling:** *Technical measures/Precautions: Ensure adequate ventilation. Provide appropriate exhaust ventilation at places where dust is formed. Safe handling advice: Use personal protective equipment. Keep away from heat and sources of ignition. Do not smoke. In case of insufficient ventilation wear suitable respiratory equipment.*

**Storage:** *Technical measures/Storage conditions: Keep containers tightly closed in a dry, cool and well-ventilated place. Store in a place accessible by authorised persons only. Incompatible products: No materials to be specially mentioned. Packaging material: No materials to be specially mentioned. Further information: Keep out of reach of children.*

#### **Transport information:**

##### ADR/RID

Class: 6.1 Item: 72 (c) TREM-CARD: 61G43c-A

Packaging group: III HI-No.: 63 Subrisks: 3 SI-No.: 2991

Hazard labels: 6.1, 3, 11

Proper shipping name: 2991 Carbamate pesticide, liquid, toxic, flammable (contains methomyl, ethanol), 6.1, 72 (c), ADR

##### IMO

Class: 6.1 UN-No: 2991 Packaging Group: III

Subrisks: 3 EmS: 6.1 - 04

Hazard labels: 6.1, 3, Marine pollutant mark

Proper shipping name: Carbamate pesticide, liquid, toxic, flammable (contains methomyl, ethanol), Class 6.1, UN 2991, PG III

##### ICAO

Class: 6.1 UN/ID No.: 2991 Packaging Group: III

Subrisks: 3 Hazard labels: 6.1, 3, 11

Packaging instructions (passenger aircraft): 611 / 60 L

Packaging instructions (cargo aircraft): 618 / 220 L

Proper shipping name: Carbamate pesticide, liquid, toxic, flammable (contains methomyl), Class 6.1, UN 2991, PG III

**Protective clothing and equipment proposed:** Eye protection: tightly fitting safety goggles or face-shield. Respiratory equipment: respirator with combination filter for

vapour/particulates. Respirator with A2B2P3 filter. Hand protection: protective rubber gloves. Skin and body protection: complete suit protecting against chemicals.

**Fire-fighting measures:** Suitable extinguishing media: Water spray, foam, dry chemical, carbon dioxide (CO<sub>2</sub>). Extinguishing media which must not be used for safety reasons: High volume water jet (contamination risk). Special protective equipment for firefighters: In the event of fire, wear self-contained breathing apparatus. Specific methods (on small fires): If area is heavily exposed to fire and conditions permit, let fire burn itself out since water may increase the area contaminated. Cool containers / tanks with spray water.

**Information on combustion products likely to be generated in the event of a fire:** In the event of fire, the formation of hydrogen cyanide, sulphur dioxide, methyl isocyanate, obnoxious, and toxic fumes must be anticipated.

**Procedures to minimise the generation of waste:** Only purchase and store quantities of product required in the short term. Do not open larger containers than is necessary for immediate use. Do not prepare spray mixture volumes greater than is necessary for immediate use

#### B.3.5.5 Emergency measures in case of an accident (IIIA 4.5)

The notifier has proposed the following:

**General procedure: Methods for cleaning up:** Soak up with inert absorbent material. If liquid has been spilt in large quantities clean up promptly by scoop or vacuum. Use approved industrial vacuum cleaner for removal. Shovel into suitable container or disposal. Environmental precautions: Do not flush into surface water or sanitary sewer system.

**Protection of emergency workers and bystanders:** Use personal protective equipment.

**First aid measures:** Inhalation: Move to fresh air. Oxygen or artificial respiration, if needed. Call a physician or Poison Control Centre immediately. Skin contact: Wash off immediately with soap and plenty of water, removing all contaminated clothes and shoes. If a person feels unwell or symptoms of skin irritation appear, consult a physician. Eye contact: Rinse immediately with plenty of water for at least 15 minutes. Keep eyes wide open while rinsing. Consult a physician. Ingestion: Do not induce vomiting. Call a physician or Poison Control Centre immediately. Never give anything by mouth to an unconscious person. Treatment: Administer atropine sulphate as antidote. Morphine, 2-PAM, and oxime therapy are contra-indicated.

#### B.3.5.6 Procedures for destruction or decontamination of the plant protection product and its packaging (IIIA 4.6)

##### a) Decontamination of Manufacturing Plant Containers

The notifier proposes, "Close and label the waste receptacles and, likewise, any uncleaned containers. Dispose of them at a suitable waste incineration plant and/or in



*accordance with the official local regulations. Where large quantities are concerned, consult the supplier. Do not allow material to contaminate ground water system. Do not contaminate surface water. Contaminated packaging: Do not re-use empty containers. Triple rinse containers. Do not contaminate ponds, waterways or ditches with chemical or used containers. If recycling is not practicable, dispose of in compliance with local regulations.”*

b) Waste Disposal

Controlled incineration is stated to be the only currently-available method.

### B 3.6 Summary of further information on the active substance and plant protection product

Information supplied adequately addresses methods for handling the active substance and plant protection products.

### B.3.7 References relied on

Annex point	Author	Year	Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or Unpublished	Data protection claimed Y/N	Owner
Annex II Tier II Section 1	Schmuckler, M E and Stattmann, M	Nov 2002	Identity, Physical and chemical properties & Further information Revision 1	Yes	DuPont

### Plant Protection Product – Methomyl 20 SL

Author	Annex point	Year	Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or Unpublished	Data protection claimed Y/N	Owner
Cosgrove, T and Stattmann, M	Annex III Tier II Section 1	Nov 2002	Identity, Physical and chemical properties & Further information Revision 1	Yes	DuPont
Stattmann, M and Ranken, D D.	Document D-1	2001	Methomyl 20% Soluble Concentrate (SL) Details of the intended uses and Conditions of Use (GAP) supported in relation to the proposed inclusion of the plant protection product	Yes	DuPont

<b>Author</b>	<b>Annex point</b>	<b>Year</b>	<b>Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or Unpublished</b>	<b>Data protection claimed Y/N</b>	<b>Owner</b>
Huby, J.P., Marquet, F.	IIIA, 4.2.c	2003	Rinsing Lannate® 20L out of Field Application Equipment DuPont de Nemours (France) S.A.S. DuPont-13768 GLP: No Published: No	N	DuPont

## **B.4 Proposals for classification and labelling**

### **B.4.1 Active substance: Methomyl**

#### Human health effects

‘Very toxic by inhalation’ (R26)

‘Toxic if swallowed’ (R25)

#### Ecotoxicological effects

N Dangerous to the environment

Very toxic to aquatic organisms (R50)

May cause long term adverse effects in the aquatic environment (R53)

### **B.4.2 Plant Protection Product: ‘Methomyl 20 SL’**

#### Human health effects

‘Toxic if swallowed’ (R25),

‘Harmful by inhalation’ (R20)

‘Risk of serious damage to eyes’ (R41).

#### Ecotoxicological effects

N Dangerous to the environment

Very toxic to aquatic organisms (R50)

May cause long-term adverse effects in the aquatic environment (R53)

### **B.4.3 References relied on**

Refer to B.6.2 and B.6.11 of the Toxicology section and B.9.2.1 of the Ecotoxicology section.

**B.5 Methods of analysis****B.5.1 Analytical methods for formulation analysis (IIA 4.1, IIIA 5.1)**  
*and***B.5.1.1 Technical active substance (IIA 4.1)**

- 1) Validation of the analytical method X1179.220.06.ES for methomyl: technical methomyl and products: Methomyl 20SL (and Lannate© 25WP).  
*Platz, S.J. (2001) DuPont Report No.: DuPont-5540.*

This method can be used to assay methomyl as manufactured or the methomyl content of Methomyl 20SL. Samples were dissolved by ultrasonication in 10% methanol/90% water (pH 2.7), treated with benzamide internal standard and filtered. Analysis was by reversed-phase HPLC (Zorbax® RX-C8, 5-µm, with a mobile phase comprising an isocratic mixture of acetonitrile / water adjusted to pH 2.7, column temperature 40°C) with quantitation by ultraviolet absorbance at 235 nm. Methomyl was determined by comparing peak area ratios of methomyl/benzamide with a standard calibration curve.

Evaluation

Validation data were presented for accuracy (recoveries), precision (%RSD), linearity (including calibration graph), and specificity (chromatographic evidence): for statistical data see Table 5.1. The report notes that in certain formulations (Methomyl 20SL included), co-formulants can appear as late eluting peaks. Reference was made to placebo formulations though no chromatograms were presented. A remedial method was provided for dealing with this, but the magnitude of the problem and its possible impact on quantitative results was not directly addressed. However, it is accepted that having publicised the problem a competent analyst would not be likely to generate misleading results.

Conclusion

The method is acceptably validated for determination of methomyl in the technical material and in Methomyl 20SL.

NB: statistical data for this method alone are summarised in Table 5.1. Acceptable, if more limited data, was also submitted for methods L30.4662 (E) / X1179.000 (P).

- 2) Method L30.4662 (E) for the determination of methomyl (*Tanya K. S. Djanegara, 1992) Document J (DuPont-5872) AMR-2267-91*

The applicant has requested that this method be retained in the Confidential section, as the details formed part of a report on analysis of impurities (DuPont-5873, below). However, method L30.4662 (E) – and its later development X1179.00 (P) used for analysis of the formulation – are significantly different from the methods for impurities; moreover, disclosure of method details will not involve disclosure of details about the impurities, so method details will not be treated as confidential material.

In method L30.4662 (E) / X1179.00 (P) the sample (technical methomyl or Methomyl 20SL) is dissolved by ultrasonication in a solution of 90% water/10% methanol and treated with a known amount of benzamide internal standard. Samples are filtered before analysis. Analysis is by reversed-phase HPLC (Zorbax<sup>®</sup> ODS column, used with a mobile phase comprising an isocratic mixture of acetonitrile / water adjusted to pH 7, column temperature 45/40°C) with quantification by ultraviolet absorbance at 254 nm. Methomyl was determined by comparing peak area ratios of methomyl/benzamide with a standard calibration curve. Method X1179.000 (P) was very similar to method L30.4662 (E): selectivity was adjusted by using a different ODS column and reducing column temperature to 40°C (from 45°C).

### Evaluation

Acceptable data were presented for precision and chromatograms were supplied demonstrating selectivity. No data were presented in the context of this method report for linearity. However, report AMR 2267-91 includes references to a later version of the L30.4662 (E) method, X1179.000 (P), developed for analysis of formulations (Methomyl 20SL included). Acceptable data for precision and accuracy was supplied for this method. See Table B.5.1.

### Conclusion

Collectively, the methods X1179.220.06.ES, L30.4662 (E) and X1179.000 (P) provide acceptable evidence that methomyl can be determined in both the technical material and in formulation Methomyl 20SL with acceptable reliability. Most complete evidence of precision, accuracy, linearity and selectivity was presented for method X1179.220.06.ES, with enough evidence for L30.4662 (E) / X1179.000 (P) to indicate that a reliable alternative is available should this be required.

## **B.5.1.2 Impurities (IIA 4.1)**

### **B.5.1.2.1 Organic impurities (excluding solvents)**

See Confidential Section – Volume 4, Annex C

### **B.5.1.2.2 Solvent impurities**

See Confidential Section – Volume 4, Annex C

## **B.5.1.3 Plant protection product (IIIA 5.1)**

### **Method(s) for relevant breakdown products, isomers, impurities and additives in the formulations**

The notifier has asserted that:

There are no impurities, isomers, or additives known to be of toxicological or environmental significance in methomyl as manufactured or in formulating

ingredients of Methomyl 20SL, which would justify the submission and disclosure of enforcement methods.

This is accepted.

## **B.5.2 Analytical methods (residue) for treated plants, plant products, foodstuffs of plant and animal origin and feeding stuffs (IIA 4.2.1, IIIA 5.2)**

*Residue definition: parent methomyl*

### **B.5.2.1 Methods for treated plants, plant products, and foodstuffs of plant origin**

#### **B.5.2.1.1 Analysis of plant commodities:**

1. deKok, A. et al. Chromatographia, 1987, Vol. 24, 469-476 (4.2.1.1/01);
2. deKok A. et al. J.AOAC Int., 1992, Vol. 75 1063-1072 (4.2.1.1/02);
3. the report of the General Inspectorate for Health Protection, The Netherlands 1996 for Netherlands Multi-Residue Method 2, Submethod 1 (4.2.1/03)
4. Dubey, L. et al. Report: DuPont-4689, 2001 (4.2.1.1/04); and
5. Merricks, L., Slaughter, C. DuPont Report No. AMR-4450-97 (4.2.1.1/05)

The applicant has cited the published method due to deKok et al. (1987) as the original study. This method entails sample extraction, SPE clean-up, and HPLC with post-column derivatisation and fluorescence detection. A study due to Dubey et al. (below) is cited as the ILV study. For regulatory purposes, the ILV study provides the more complete demonstration of method performance, while the above five papers / reports (with their different emphasis) address only selected aspects of validation.

Reports 1-2 and 4 above describe essentially the same method as covered by the ILV report below; while the report of the General Inspectorate for Health Protection, The Netherlands (1996) provides a summary of the method and previously published data.

The above papers include data for selected plant commodities not covered by the ILV study: this data is included in Table B.5.2.

The method reported by Merricks and Slaughter (4.2.1.1/05) was used in a study investigating the dissipation of foliar residues of methomyl from lettuce leaves. Samples were extracted by surface-washing with aqueous detergent; clean-up was by solvent partition (dichloromethane), and analysis by reversed phase HPLC with UV detection (233nm). Chromatograms, calibration details and recovery data were included; the recovery data is included in Table B.5.2.

#### **B.5.2.1.2 Independent Laboratory Validation: Netherlands Multi-Residue Method 2, Submethod 1: methomyl / various crops L. Dubey et al. 2002, DuPont-6309**

Samples were extracted into solvent, subjected to SPE cartridge clean-up, and analysed by reversed phase HPLC with post-column derivatisation and fluorescence detection. Quantification was by an internal standard procedure.

Samples were extracted by maceration and solvent-partition procedures. The extraction solvent was adapted to suit the water and fat content of samples. After clean-up using an aminopropyl SPE cartridge, extracts were reconstituted in internal standard (trimethacarb) solution for HPLC analysis (Zorbax C8 column at 30°C, water / acetonitrile gradient). Post-column derivatisation used the phthalaldehyde / mercaptoethanol reaction with fluorescence detection (Ex. 330nm, Em. 466nm).

A confirmatory method using HPLC-MS was described (SIM monitoring m/z 162.6 – note: m/z for  $[M+H]^+$  = 163.2).

Validation data for various crops (primary and confirmation methods) is summarised in Table B.5.2

### Evaluation

Primary and confirmatory methods were acceptably validated for precision, accuracy (recovery), linearity and specificity to an LOQ of 0.01mg/kg (methomyl parent) for most crops. No data for a leafy green crop were included in the ILV report, although limited data due to deKok (4.2.1.1/02) are available. Although the deKok et al. and Dubey et al. sources do not represent exact duplications of the methodology or crops covered, there is enough fundamental similarity between methods and diversity of sample types to regard the method as independently validated for the three major crop groups. Multi-residue analysis of carbamates by the HPLC-fluorescence method (deKok et al., 4.2.1.1/01) was limited by poor resolution particularly of the early-eluting carbamates, of which methomyl was one. Recovery data for the latter work was done using isocratic rather than gradient elution as would be preferred for an efficient multi-residue analysis.

The utility of the HPLC-UV method of Merricks and Slaughter (4.2.1.1/05) for fully homogenised green-leaf crops is uncertain: the method is unlikely to be as selective as the gradient HPLC-fluorescence method, for which acceptable data for spinach were reported.

The HPLC-MS confirmatory method suffers from having only one strong ion with m/z >100 ( $[M+H]^+$  = 163.2) but the method is very selective for several crops. Paired with the selectivity of the HPLC-fluorescence method, acceptable method specificity is available for primary / confirmatory monitoring. The publications of deKok et al. (4.2.1.1/01) refer to published GC methods for carbamates, although no data for GC analysis of methomyl in crops formed part of this dossier submission.

### Conclusion

Monitoring methods for crops are acceptably validated for parent methomyl.

## **B.5.2.2 Methods for foodstuffs of animal origin**

No residues were found in ruminant metabolism and feeding studies or in a poultry study (45x feeding level) and there will be no MRLs for produce of animal origin.

A method was supplied (as supplementary information) for meat, milk, eggs and fat: this will not be required for post-monitoring and has not been evaluated. No ILV is required.

### **B.5.3 Analytical methods (residue) in soil, water and air (IIA 4.2.2 to 4.2.4, IIIA 5.2)**

#### **B.5.3.1 Residues in soil (IIA 4.2.2) and water (IIA 4.2.3)**

Methods were reported for both soil and sediment, although validation data for sediment are limited. The reports due to Ruhl et al. report methods for both soil and ground water samples.

- 1) Ruhl J.C., DuPont Report No. AMR 2311-92 (4.2.2.1/01)  
Soil (and ground water) by HPLC-fluorescence
- 2) Ruhl J.C. et al. DuPont Report No. AMR 2759-93 (4.2.2.1/02)  
Soil (and ground water) by ELISA, validated by HPLC-fluorescence

Soil samples were extracted with methanol, solvent swapped (water) and cleaned up using SPE (C18). Water samples were applied directly to the SPE cartridges. Methomyl residues were eluted with acetone and reconstituted in HPLC mobile phase.

Analysis was by HPLC-fluorescence (described above), with chromatography modified to suit the extracts: Zorbax ODS 2 column; isocratic mobile phase (acetonitrile:water). The LOQ was 0.001 mg/kg. Validation data are recorded in Table B.5.3.

An enzyme-linked immunosorbent assay method (ELISA) was validated for soil and the HPLC-fluorescence analysis was used to confirm the ELISA results and validate ELISA as a standalone method. Soil samples were prepared for ELISA by extraction with buffer (acetone:phosphate 90:10), followed by filtration and dilution as appropriate. Optical density measurements were by UV absorbance.

Validation data are summarised in Table B.5.3

- 3) Determination of ten carbamate pesticides [in sediment] by HPLC-IS-MS, HPLC-TSP-MS

*Honing M., et al. J. Chromatography 1996 Vol. 733 283-294 (4.2.2.1/04)*

Sediment samples were sieved, freeze-dried, and extracted with acetone-dichloromethane using a Soxhlet extractor. The extract was evaporated to dryness, reconstituted in hexane, and subjected to SPE column clean-up (aminopropyl). Following elution (25:75 acetone dichloromethane) samples were evaporated and reconstituted in 20:80 methanol:water. Analysis was by reversed phase HPLC (Licrosphere 60 RP-select B base-deactivated phase, methanol/water gradient) with thermospray MS detection. Selected ion monitoring MS detection was used for the  $[M+Na]^+$  ion.

Limited validation data are recorded in Table B.5.3



### Evaluation

The HPLC-fluorescence method (described above for crops) was acceptably validated for soil samples with an LOQ of 0.001mg/kg. An ELISA method was also validated by comparison against the HPLC-fluorescence method to an LOQ of 0.025mg/kg. ELISA cross-reactivity with methomyl oxime, oxamyl, aldicarb, thiodicarb and a range of other pesticides was investigated: only thiodicarb showed significant cross-reactivity (50%). The HPLC and ELISA methods were shown to be statistically equivalent. From a regulatory standpoint and where a single method is not acceptable the data demonstrate that primary and confirmatory methods are available for soil. The HPLC-MS method applied to sediment by Honing et al. demonstrates the potential of this method for sediment samples but since statistical (and chromatographic) evidence was limited the method cannot be described as fully validated for regulatory purposes. However, such a method of analysis is not specified in the legislation as an actual regulatory requirement.

### Conclusion

Monitoring methods for soil are acceptably validated.

#### **B.5.3.2 Residues in water**

- 1) Ruhl J.C., DuPont Report No. AMR 2311-92 (4.2.2.1/01)  
Soil (and ground water) by HPLC-fluorescence
- 2) Ruhl J.C. et al. DuPont Report No. AMR 2759-93 (4.2.2.1/02)  
Soil (and ground water) by ELISA, validated by HPLC-fluorescence

The methods used for water samples were the essentially same as those described above for soil, with the exception that no initial extraction was involved. Validation data for ground water and surface waters is summarised in Table B.5.3.

- 3) Munch J.W. (ed) EPA Method 531.1 Rev. 3.1 pp531.1-1 to 531.1-24  
(4.2.3.1/03)

The HPLC-fluorescence method applied elsewhere to food crops, soil and water samples is here applied to a multi-residue determination of carbamates (including methomyl) in synthetic ground and surface waters (see Table B.5.3). Water samples were buffered to pH 3 (monochloroacetic acid), filtered and analysed without clean-up by gradient HPLC-fluorescence. Matrix interferences are referred to, but not illustrated with chromatograms. The synthetic waters analysed were detailed: these waters were relatively 'clean' and are seen as supplementary to the data obtained from the natural surface water sourced from Florida. Validation data are limited but the method is accepted as validated (for methomyl parent) based on separate data for soil and water. The relevant data are summarised in Table B.5.3.

### Evaluation

The methods described by Ruhl et al. and based on the HPLC-fluorescence method were acceptably validated for precision, accuracy, linearity and specificity to an LOQ of 0.0001mg/kg. As noted under analysis of crops, use of HPLC-fluorescence

for multi-residue analysis of carbamates may be limited by the resolution available for the more polar carbamates, which in turn will limit the number of analytes in a single standard mixture. Toxicological studies have established a concentration limit for drinking water of 15 µg/litre: the method is capable of monitoring to this level.

### Conclusion

Monitoring methods for methomyl parent in ground and surface waters were acceptably validated.

### **B.5.3.3 Residues in air (IIA 4.2.4)**

Bacher R., DuPont Report No. AMR-4564 (4.2.4.1/01)

Samples were collected and desorbed from ORBO-44 adsorption tubes (two in series) packed with XAD-2 porous polymer. Sample residues were desorbed with acetonitrile. Analysis was by reversed phase HPLC (Luna RP-C18 column, acetonitrile / water gradient) with MS detection and external calibration. Residues were confirmed by conversion of methomyl to the oxime, followed by HPLC-MS analysis ( $[M+H]^+$   $m/z = 106$ ).

Validation data are presented in Table B.5.3.

### Evaluation

The method was acceptably validated for precision, accuracy, linearity and specificity. Analysis by HPLC-MS for both methomyl and its oxime were acceptably validated. The LOQ for methomyl was 0.58 µg/m<sup>3</sup> air validated up to 5.8 µg/m<sup>3</sup> air without breakthrough. The necessary extremes of temperature and humidity were covered (Table B.5.3). This report also presents data for oxamyl, not covered by this evaluation. The HPLC-fluorescence method could most likely be used for confirmation of residues in air, though no data were presented.

### Conclusion

A monitoring method for air was acceptably validated.

### **B.5.4 Analytical methods (residue) in human and animal tissues and fluids (IIA 4.2.5, IIIA 5.2)**

Analytical method for the determination of oxamyl and methomyl in human body fluids. Bacher, R. (2001a); DuPont-5133 (4.2.5.1/01)

Body fluid (urine or blood serum) was extracted with ethyl acetate. Extracts were concentrated, filtered through anhydrous sodium sulphate, and further concentrated by evaporation. NaCl:Na<sub>2</sub>SO<sub>4</sub> (1:1, w/w) and cyclohexane were added in succession, followed by filtration. Cleanup was by gel permeation chromatography (BioBeads SX-3 resin, ethyl acetate / cyclohexane (1:1, v/v) eluent). The GPC fraction was concentrated and hydrolysed (water / 1 N NaOH) to convert methomyl to the oxime derivative. Following acidification, the oxime was

extracted with dichloromethane and the solvent evaporated. The dry residue was dissolved in ethyl acetate. Residues of methomyl oxime were determined by GC/MS (5%-phenyl-95%-dimethylpolysiloxane column, temperature gradient and helium carrier gas). Ion trap electron impact (EI) mass spectrometric detection was used with  $m/z = 88$  used for quantification, and  $m/z = 58$ , and  $m/z = 105$  used for confirmation. Quantification was by external standards prepared from a certified reference standard of methomyl oxime. Standards were not matrix-matched. Residue results were expressed as methomyl equivalents. GC/pulsed flame-photometric detection (GC/PFPD, used in sulphur mode) was used for confirmation.

Validation data are summarised in Table B.5.4

### Evaluation

Determination of methomyl by the GC/MS method was acceptably validated in human blood serum and human urine to LOQs of 0.01mg/kg. Only limited data were presented for the GC/PFPD method, but adequate detection at the LOQ was demonstrated. Some potential for matrix interference was noted by the applicant for both sample types in both methods. The problem appeared more marked for the GC/PFPD method, but in the samples studied, adequate resolution was achieved at the 0.01mg/kg level. The applicant notes however that variations in the matrix and/or clean-up efficiency may lead to more obstructive interferences in some samples.

The applicant has remarked that no residues of methomyl or oxime are anticipated in human tissues or body fluids: this position is supported by metabolism data and is accepted.

### Conclusion

Analytical methods for human serum and urine may, in some samples, suffer interference effects at the validated LOQ of 0.01mg/kg. An ARfD and AOEL of 0.005mg/kg / bw / day has been established for serum. The validated LOQ of 0.01mg/kg is accepted as adequate for analysis of exposure cases. The method does not discriminate between methomyl and methomyl oxime: refer to following Section.

## **B.5.5 Conclusions: methods of analysis**

Acceptably validated methods of analysis are available for the active ingredient both in the technical material and in the product Methomyl 20SL.

Residues monitoring in plant commodities is acceptably supported by variations on the HPLC-Fluorescence method reported by deKok et al., and confirmatory methods using GC-MS were reported. Acceptable LOQs of 0.01mg/kg have been demonstrated for these methods and while original and ILV studies do not duplicate each other exactly and statistical data is limited for some crops there is sufficient breadth and depth of data to give assurance that methods will be widely reliable and robust.

Environmental monitoring of soil also draws on the HPLC-Fluorescence method of deKok et al. An LOQ of 0.001mg/kg was validated for soil. ELISA was also validated for soil with an LOQ of 0.025mg/kg. The analysis of sediment samples by HPLC-MS to an LOQ of 0.05mg/kg has also been demonstrated but the method was not fully validated. However, such a method of analysis is not an actual regulatory requirement.

Water samples were also analysed using the HPLC-Fluorescence method of deKok et al. An LOQ of 0.0001mg/kg was validated for ground water. Using the same method, validated LOQs of 0.00025mg/kg were achieved for a Florida-sourced surface water and two synthetic surface waters were validated to 0.0025mg/kg.

Air was analysed by HPLC-MS. The LOQ for methomyl was 0.58  $\mu\text{g}/\text{m}^3$  air (determined as the oxime, expressed as parent methomyl) validated up to 5.8  $\mu\text{g}/\text{m}^3$  air without breakthrough.

Human serum and urine were analysed by HPLC-MS each to a validated LOQ of 0.01mg/kg (determined as the oxime, expressed as parent methomyl). Limited validation data were reported for a confirmatory analysis using GC/PFPD. The method does not discriminate between methomyl and methomyl oxime. Results reported by this method could therefore exaggerate estimated exposure to methomyl parent. In the context of a diagnostic or therapeutic analysis this is not expected to be important.

Most methods of analysis target parent methomyl only. The review has concluded that for food-stuff matrices and environmental samples the residue definition should be methomyl parent. Primary monitoring will therefore be for parent methomyl.

For all sample types, primary methods were acceptably supported by confirmatory methods. Although not demonstrated for all sample types, it is likely that HPLC-MS (or GC-MS) will provide acceptable alternative methods of confirmation where required.

In summary: methods have been evaluated for the analysis of methomyl in plant food commodities, environmental samples and human fluids and were found to be acceptably validated to the necessary limits of quantification. Methods were also supplied for analysis of food products of animal origin, but as these will not be needed for monitoring they have not been considered under this evaluation.

### **Overall conclusion**

Based on methods of analysis, methomyl can be recommended for Annex I inclusion.

Table B.5.1 Summary of method description and validation (active substance and plant protection product)

Substrate	Analyte	Accuracy (%) (mean recovery)	Interference	Precision - reproducibility (n)	Precision - repeatability RSD (%) (n)	Linearity demonstrated (range mg/l)	Reference
<b>Technical material</b>  Method: X1179.220.06.ES	Methomyl	100.0 (2% fort.) (2) 99.9 (10% fort.) (2)	None	Not required	0.48 (8)	750 – 1500	DuPont-5540 (5.1.1/01)
<b>Formulation:</b> <b>Methomyl 20SL</b> Method: X1179.00 (P) (formerly L30.4662 (E)  X1179.220.06.ES	Methomyl	100.1 (6.6% fort) (2) 99.8 (10% fort.) (2)  100.1 (2% fort) (-) 99.8 (10% fort.) (-)	Some late-running interferences. See text	0.26 (8) 0.30 (8) Pooled CV = 0.27% (16)	0.35 (4) 0.18 (4) 0.28 (4) 0.25 (4)  Mean = 19.31% RSD = 0.31% (-)	Not Det. But linearity expected to be similar to X1179.220.06.ES  See Tech Material above	DuPont-5873 Report:: AMR 2267-91, plus Letter One

Acceptable (non-GLP) data was presented demonstrating precision ('pooled CV' = 0.27% for two operators on two days analysing two different technical materials, n = 4 for each batch). Accuracy was acceptably demonstrated for technical materials spiked at two levels).

Table B.5.2 Summary of method description and validation (treated plants, plant products, foodstuffs, feedingstuffs)

*Analyte = methomyl. Primary method = HPLC / Fluorescence. Confirmation method = HPLC-MS*

Substrate	Limit of quantification (mg/kg)	Recovery fortification level (mg/kg)	Mean recoveries % (n) (RSD%)	Repeatability %RSD (n) *	Linearity demonstrated	Interferences	HPLC-MS confirmation values (mean)	Reference
<b>Wheat grain</b> HPLC-fluorescence HPLC-MS	0.01	0.01 0.10	84 (5) (15) 91 (5) (9)	13 (10)	Yes	Primary: none	91, 94 (93) 81, 89 (85)	<u>DuPont 6309</u>
<b>Grain</b> HPLC-fluorescence	0.05**	0.05 0.5	98 (5) (1.4) 101 (5) (1.4)	-	No	None reported	-	deKok et al. 1987 (4.2.1.1/01)
<b>Carrot</b> HPLC-fluorescence	0.05	0.2	87 (5) (2.8)	-	No	None reported	-	deKok et al. 1987 (4.2.1.1/01)
<b>Cauliflower</b> HPLC-fluorescence	0.05	0.2	80 (5) (2.1)	-	No	Isocratic separation. Some interferences apparent	-	deKok et al. 1987 (4.2.1.1/01)
<b>Grape berries</b> HPLC-fluorescence HPLC-MS	0.01	0.01 0.1 0.01 0.10	82 (6) (6) 75 (5) (7) 89 (2) 78 (2)	7 (11) 13 (4)	Yes	Primary: Minor (negligible)  Conf: none	- - 82, 84 (83) 70, 70 (70)	DuPont 4689  <u>DuPont 6309</u>
<b>Grape juice</b> HPLC-fluorescence	0.01	0.01 0.10	81 (5) (8) 76 (5) (7)	8 (10)	Yes	Primary: none	- -	DuPont 4689
<b>Grape wine</b> HPLC-fluorescence HPLC-MS	0.01	0.01 0.10 0.01 0.10	99 (5) (4) 95 (5) (2) 105 (2) 90 (2)	4 (10) 9 (4)	Yes	Primary: none  Conf: none	- - 94, 95 (95) 86, 85 (86)	DuPont 4689  <u>DuPont 6309</u>
<b>Grape raisins</b> HPLC-fluorescence HPLC-MS	0.01	0.01 0.10 0.01 0.10	99 (5) (10) 99 (5) (5) 89 (2) 84 (2)	7 (10) 11 (4)	Yes	Primary: none  Conf: none	- - 98, 84 (91) 74, 78 (76)	DuPont 4689  <u>DuPont 6309</u>

Substrate	Limit of quantification (mg/kg)	Recovery fortification level (mg/kg)	Mean recoveries % (n) (RSD%)	Repeatability %RSD (n) *	Linearity demonstrated	Interferences	HPLC-MS confirmation values (mean)	Reference
<b>Tomato fruit</b> HPLC-fluorescence HPLC-MS	0.01	0.01 0.10	87 (5) (10) 87 (5) (10)	9 (10)	Yes	Primary: none Conf: none	89, 99 (94) 89, 88 (89)	<u>DuPont 6309</u>
<b>Tomato juice</b> HPLC-fluorescence	0.01	0.01 0.10	88(5) (3) 79 (5) (6)	7 (10)	Yes	Primary: none	- -	<u>DuPont 6309</u>
<b>Tomato puree</b> HPLC-fluorescence HPLC-MS	0.01	0.01 0.10	80 (5) (6) 94 (5) (7)	10 (10)	Yes	Primary: Minor (negligible) Conf: none	93, 93 (93) 85, 95 (90)	<u>DuPont 6309</u>
<b>Peanuts (nutmeat)</b> HPLC-fluorescence HPLC-MS	0.01	0.01 0.10	83 (5) (12) 81 (5) (3)	9 (10)	Yes	Primary: none Conf: none	86, 97 (92) 94, 86 (90)	<u>DuPont 6309</u>
<b>Citrus peel</b> HPLC-fluorescence	0.01	0.01 0.10	101 (5) (4) 94 (5) (7)	7 (10)	Yes	Primary: IS not measurable.	- -	<u>DuPont 6309</u>
<b>Citrus pulp</b> HPLC-fluorescence HPLC-MS	0.01	0.01 0.10	86 (5) (16) 88 (5) (5)	10 (10)	Yes	Primary: none Conf: none	77, 82 (80) 84, 85 (85)	<u>DuPont 6309</u>
<b>Spinach</b> HPLC-fluorescence	0.05	0.05 0.5	80 (1) (-) 81 (1) (-)	-			-	deKok et al. 1987 (4.2.1.1/01)
<b>Beans</b> HPLC-fluorescence	0.05	0.05 0.5	77 (1) (-) 86 (1) (-)	-			-	deKok et al. 1987 (4.2.1.1/01)
<b>Lettuce</b> HPLC-UV	0.001 µg/cm <sup>2</sup>	0.001 0.01 0.1	92 (7) (1.4) 97 (7) 4.4 94 (7) (2.2)	-	Yes	None (surface-wash only).	-	DuPont AMR-4450-97 (4.2.1.1/05)

\* Replicate extractions and analysis. Recovery level not reported.

\*\* Validated LOQ; deKok paper reports LOQ typical LOQs of 0.005 – 0.01mg/kg for most crops

Table B.5.3 Summary of method description and validation (environmental samples)

Substrate (method)	Analyte	Limit of quantification (mg/kg)	Recovery fortification level (mg/kg)	Mean recoveries % (range) (n)	Repeatability %RSD (n)	Linearity demonstrated	Interference	Reference
<b>Soil</b> HPLC-Fluorescence	Methomyl	0.001	0.001 - 1.0 <sup>a</sup>	99 (73-116) (71)	9 (71)	Yes	None	DuPont AMR 2311-92 V2 p195
<b>Soil</b> ELISA, confirmed by HPLC-fluorescence	Methomyl	0.01 <sup>b</sup>	0.025 – 20 <sup>a</sup>	103 (92-113) (9)	-	Yes	Cross-reaction with thiodicarb	DuPont AMR 2759-93
<b>Sediment</b> HPLC-TSP-MS	Methomyl	0.05	0.05 0.5	- <sup>c</sup> (90-100) (5)	-	See text	None	Honing M., et al. (4.2.2.1/04)
<b>Ground water</b> HPLC-Fluorescence	Methomyl	0.0001	0.0001 - 0.002 <sup>a</sup>	90 (67-110) (143)	11 (143)	Yes	None	DuPont AMR 2311-92 V2 p188
<b>Ground/surface water</b> HPLC-Fluorescence	Methomyl		0.0025 <sup>d</sup> 0.0025 <sup>e</sup>	98 (-) (7-8) 105 (-) (7-8)	6.9 (7-8) 9.5 (7-8)	See text	Yes. See text.	Munch J.W. (ed) (4.2.3.1/03)
<b>Surface water (Florida)</b> HPLC-Fluorescence, Conf. by ELISA	Methomyl	0.00025	0.00025 – 0.6 <sup>a</sup>	95 (79-104) (32)	6.9 (32)	Yes	None	DuPont AMR 2759-93
<b>Air</b> HPLC-MS	Methomyl							DuPont-4564
	25°C 30% rel. hum.	0.58µg/m <sup>3</sup>	0.58µg/m <sup>3</sup> 5.8µg/m <sup>3</sup>	98 (89-107) (5) 79 (67-93) (5)	8 (5) 14 (5)	Yes	None	
	35°C 100% rel. hum.		0.58µg/m <sup>3</sup> 5.8µg/m <sup>3</sup>	95 (85-107) (5) 104 (97-118) (5)	10 (5) 8 (5)			

a. Statistics for collated data for a range of recovery levels.

b. Estimated from calibration, i.e. not validated.

c. Mean not reported. Unclear whether this represents pooled results for 0.05 and 0.5 levels.

d. Synthetic Water 1: Absopure Nature Artesian Spring Water (Absopure Water Company), Plymouth, Michigan.

e. Synthetic Water 2: Reagent grade water fortified with fulvic acid (1mg/litre)



Table B.5.4 Summary of method description and validation (human fluids)

Substrate	Analyte	Limit of quantification (mg/kg)	Recovery fortification level (mg/kg)	Recoveries % range (mean)	Repeatability RSD (%) (n)	Linearity demonstrated	Interferences	Ref.
<b>Blood serum</b> GC-MS, conf by GC/PFPD	Methomyl	0.01	0.01	76 – 116 (89)	18 (5)	yes	See text.	DuPont-5133
			0.1	77 – 95 (85)	9 (5)			
<b>Urine</b> GC-MS, conf by GC/PFPD	Methomyl	0.01	0.01	86 – 108 (95)	9 (5)	yes	See text.	DuPont-5133
			0.1	79 – 105 (94)	12 (5)			

**B.5.6 References relied on**

<b>Annex point</b>	<b>Author</b>	<b>Year</b>	<b>Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or Unpublished</b>	<b>Data protection claimed Y/N</b>	<b>Owner</b>
Bacher, R.	IIA, 4.2.4.1./01	2000	Development and validation of an analytical method for the development of oxamyl and methomyl in air PTRL Europe DuPont-4564 GLP: Yes Published: No	Y	DuPont
Bacher, R.	IIA, 4.2.5.1./01	2001a	Analytical method for the determination of oxamyl and methomyl in human body fluids PTRL Europe DuPont-5133 GLP: Yes Published: No	Y	DuPont
Blaisdell, C.T.	IIA, 4.1.1./01	1990	Methomyl technical (Lannate□) product identity and composition DuPont Experimental Station AMR 1735-90 Information superseded by reports or publications submitted. Published: No	Y	DuPont
Blaisdell, C.T.	IIA, 4.1.1./02	1990	Methomyl technical (Lannate□) product identity and composition DuPont Experimental Station AMR 1735-90 Information superseded by reports or publications submitted. Published: No	Y	DuPont
deKok, A., Hiemstra, M.	IIA, 4.2.1.1./02	1992	Optimization, automation, and validation of the solid-phase extraction cleanup and on-line chromatographic determination of N-methylcarbamate pesticides in fruits and vegetables Inspectorate for Health Protection/Food Inspection Service, Pesticide Analysis Department, The Netherlands J. AOAC Int., Vol. 75, pp. 1063-1072 GLP: No Published: Yes	N	Authors

Annex point	Author	Year	Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or Unpublished	Data protection claimed Y/N	Owner
deKok, A., Hiemstra, M., Vreeker, C.P.	IIA, 4.2.1.1./01	1987	Improved cleanup method for the multiresidue analysis of N-methylcarbamates in grains, fruits and vegetables by means of HPLC with postcolumn reaction and fluorescence detection Inspectorate for Health Protection/Food Inspection Service, Pesticide Analysis Department, The Netherlands Chromatographia, Vol. 24, pp. 469-476 GLP: No Published: Yes	N	Authors
Djanegara, T.K.S.	IIA, 4.1.1./02	1992	Technical grade methomyl: analysis and certification of product ingredients DuPont Experimental Station AMR 2267-91 GLP: Yes Published: No	Y	DuPont
Djanegara, T.K.S.	IIA, 4.1.2./02	1992	Technical grade methomyl: analysis and certification of product ingredients DuPont Experimental Station AMR 2267-91 GLP: Yes Published: No	Y	DuPont
Dubey, L., Steiner, C., Mattou, H.	IIA, 4.2.1.1./06.	2002	Method validation of the Netherlands multi-residue method 2 (MRM-2, submethod 1: N-methylcarbamate pesticides) for the determination of methomyl in dry, high water, high fat and high acid content crops Battelle Europe-Centre de Recherche de Geneve DuPont-6309 GLP: Yes Published: No	Y	DuPont
Dubey, L., Steiner, C., Mattou, H.	IIA, 4.2.1.1./04	2001	Method validation for the determination of methomyl residues in different grape processing products Battelle Europe-Centre de Recherche de Geneve DuPont-4689 GLP: Yes Published: No	Y	DuPont

Annex point	Author	Year	Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or Unpublished	Data protection claimed Y/N	Owner
General Inspectorate for Health Protection, The Netherlands	IIA, 4.2.1.1./03	1996	Pesticides analysed with HPLC-procedures, submethod 1 (N-methylcarbamates) General Inspectorate for Health Protection Ministry of Public Health, Welfare, and Sport, Multi-Residue Method 2, Part 1, pp. 1-10 GLP: No Published: Yes	N	Authors
Honing, M., Riu, J., Barcelo, D., Van Baar, B.L.M, and Brinkman, U.A.TH.	IIA, 4.2.2.1./04	1996	Determination of ten carbamate pesticides in aquatic and sediment samples by liquid chromatography-ion spray and thermospray mass spectrometry Department of Environmental Chemistry of Spain; Departments of Organic and Analytical Chemistry of the Netherlands J. Chromatogr. Vol. 733, pp. 283-294 GLP: No Published: Yes	N	Authors
Kennedy, C.M.	IIA, 4.3.2.1./03	1991	Field soil dissipation of Lannate □ L insecticide - a 1991 study DuPont Experimental Station, Morse Laboratories, Inc. AMR 1921-91 GLP: Yes Published: No	Y	DuPont
Kennedy, C.M.	IIA, 4.3.2.1./04	1992	Field soil dissipation of Lannate □ L insecticide, a 1991 study Morse Laboratories, Inc., DuPont Experimental Station AMR 1921-91, Supplement No. 1 GLP: Yes Published: No	Y	DuPont
Kennedy, C.M.	IIA, 4.3.2.1./05	1994	Field soil dissipation of Lannate □ L insecticide, a 1991 study Morse Laboratories, Inc., DuPont Experimental Station AMR 1921-91, Supplement No. 2 GLP: Yes Published: No	Y	DuPont
Kennedy, S.M.	IIA, 4.3.2.1./01	1989	Field soil dissipation of Lannate □ L insecticide Morse Laboratories, Inc. AMR 1215-88 GLP: Yes Published: No	Y	DuPont

Annex point	Author	Year	Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or Unpublished	Data protection claimed Y/N	Owner
Kennedy, S.M.	IIA, 4.3.2.1./02	1990	Field soil dissipation of Lannate <sup>®</sup> insecticide Morse Laboratories, Inc., DuPont Experimental Station AMR 1215-88, Supplement No. 2 GLP: Yes Published: No	Y	DuPont
Merricks, D.L., McNeal, H.R.	IIA, 4.3.2.1./06	1998	Dissipation of dislodgeable foliar and soil residues of methomyl from grapes following application of Lannate <sup>®</sup> SP insecticide in the U.S.A. - season 1997 Agriseach, Inc. AMR 4449-97 GLP: Yes Published: No	Y	DuPont
Merricks, L., Slaughter, C.	IIA, 4.2.1.1./05	1998	Dissipation of dislodgeable foliar residues of methomyl from lettuce following application of Lannate <sup>®</sup> SP insecticide in the U.S.A. - season 1997 Agriseach, Inc. AMR 4450-97 GLP: Yes Published: No	Y	DuPont
Munch, J.W. (ed.) for US Environmental Protection Agency	IIA, 4.2.3.1./03	1995	Measurement of N-methylcarbamoyloximes and N-methylcarbamates in water by direct aqueous injection HPLC with post column derivatization National Exposure Research Laboratory U.S. Environmental Protection Agency EPA Method 531.1., Revision 3.1, pp. 531.1-1 to 531.1-24 GLP: No Published: Yes	N	Authors
Rühl, J.C.	IIA, 4.2.2.1./01	1995	A small-scale prospective groundwater monitoring study for methomyl: analytical summary Morse Laboratories, Inc., Lancaster Laboratories AMR 2311-92, 2 volumes & Supplement 1 GLP: Yes Published: No	Y	DuPont

<b>Annex point</b>	<b>Author</b>	<b>Year</b>	<b>Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or Unpublished</b>	<b>Data protection claimed Y/N</b>	<b>Owner</b>
Rühl, J.C. Baer, C.S., Kennedy, C.M., Wetherington, J.D.	IIA, 4.2.2.1./02	1994	HPLC validation of the ELISA detection method for the determination of methomyl in water and soil samples DuPont Experimental Station, Morse Laboratories, Inc. AMR 2759-93 GLP: Yes Published: No	Y	DuPont
Rühl, J.C.	IIA, 4.2.3.1./01	1995	A small-scale prospective groundwater monitoring study for methomyl: analytical summary (Appendix X) Morse Laboratories, Inc., Lancaster Laboratories AMR 2311-92, 2 volumes GLP: Yes Published: No	Y	DuPont
Rühl, J.C. Baer, C.S., Kennedy, C.M., Wetherington, J.D.	IIA, 4.2.3.1./02	1994	HPLC validation of the ELISA detection method for the determination of methomyl in water and soil samples DuPont Experimental Station, Morse Laboratories, Inc. AMR 2759-93 GLP: Yes Published: No	Y	DuPont
Rühl, J.C.	IIA, 4.3.1.1./02	1998	Enforcement method for the determination of methomyl in dry and watery crop matrices DuPont Experimental Station, Morse Laboratories, Inc., Alta Laboratories AMR 3015-94, Revision No. 2 GLP: No Published: No	Y	DuPont
Silveira, E.J	IIA, 4.1	1991	Methomyl technical (Lannate <sup>®</sup> ) product identity and composition DuPont Experimental Station AMR 1735-90, Supplement No. 1 Information superseded by reports or publications submitted. Published: No	Y	DuPont
Weidenauer, M., Dubey, L., Françon, B., Larcinese, J.P.	IIA, 4.3.1.1./04	1998	Method validation for the determination of methomyl residues in different crops by HPLC (with post-column derivatization fluorescence detection) Battelle Europe-Centre de Recherche de Geneve AMR 4258-96 GLP: Yes Published: No	Y	DuPont

**Plant Protection Product – Methomyl 20 SL**

<b>Author</b>	<b>Annex point</b>	<b>Year</b>	<b>Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or Unpublished</b>	<b>Data protection claimed Y/N</b>	<b>Owner</b>
Jourdainne, C.	IIIA, 5.1.1/02	1999	Physical, chemical and technical properties of DPX-X1179 (Methomyl) 200 g/liter soluble concentrate insecticide formulation DuPont de Nemours (France) S.A. DuPont-3094 GLP: Yes Published: No	N	DuPont
Platz, S.J.	IIIA, 5.1.1./01	2001	Validation of the analytical method for determination of the active ingredient methomyl (X1179) in the end-use products: Lannate <sup>®</sup> 25WP and Lannate <sup>®</sup> 20L DuPont <sup>®</sup> Stine-Haskell Research Center DuPont-5540 GLP: Yes Published: No	No	DuPont

## B.6 TOXICOLOGY AND METABOLISM

Methomyl is a carbamate that inhibits cholinesterase activity (Fig 6.1).

### Technical specification of the batches used in the toxicity tests

The purity of the technical material used in the toxicity studies ranged from 92-100% (several studies used material of unspecified purity). The purity of the test material in the genotoxicity studies, the long-term feeding studies and the reproduction studies ranged between 97-99%. Five batch analysis of the current technical grade material for both the DuPont source and for the Bayer/Aventis source (See Volume 1, Section 1) indicate that the impurity profiles are similar (nominal purity 98.7%). The notifier has stated that chemical manufacturing process is the same for both of these sources and there have been no significant changes to the process. Therefore, it is assumed that the current technical material reflects the material tested in the toxicity studies.

### Potential for reactivation of cholinesterase during sampling

Data from the studies submitted indicate that the inhibition of cholinesterase by methomyl is rapid and reversible following bolus dosing (acute gavage, capsule and dietary administration). The repeat dose feeding studies indicate that relatively high levels of methomyl can be tolerated without any detectable effects on cholinesterase activity. However, the time of sampling and assay are critical in the evaluation of the anticholinesterase activity. Full details of sampling and cholinesterase assay procedures were not always available; in such cases, or where 'best practice' does not appear to have been followed, this is identified in the text.

### Criteria used to evaluate inhibition of cholinesterase activity

The mechanism of action of methomyl is the inhibition of acetylcholinesterase. Most of the toxicity studies measured cholinesterase activity in plasma, erythrocytes (RBC) and brain as a surrogate for disruption of cholinergic neurotransmission. Assessment of the adversity of cholinesterase inhibition at any particular dose level has been performed in a hierarchical manner, using the guiding criteria presented below:

- i) Non-cholinergic toxicity:- is considered as for all pesticides.
- ii) Clinical signs:- evidence of altered cholinergic neurotransmission is treated as adverse. If there are no clinical signs, cholinesterase inhibition is considered.
- iii) Brain acetylcholinesterase:- inhibition by  $\geq 20\%$  which is statistically significant ( $p < 0.05$ ) is considered adverse if it fits a dose or time related trend. Inhibition of  $< 20\%$  is not considered adverse. Erythrocyte acetylcholinesterase:- inhibition of  $\geq 20\%$  which is statistically significant ( $p < 0.05$ ) is considered adverse if it fits a dose or time related trend. Inhibition of  $< 20\%$  is not considered adverse. The most sensitive of these two end-points was chosen when determining the NOAEL for a study.
- iv) Plasma butyrylcholinesterase:- is generally considered only as a marker of exposure, unless no other cholinesterase measurements have been performed.

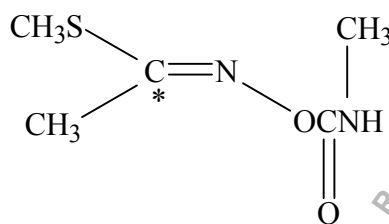


Inhibition of  $\geq 20\%$  which is statistically significant ( $p < 0.05$ ) would then be considered as an indication of adversity if it fits a dose or time related trend. Inhibition of  $< 20\%$  is not considered adverse.

However, in dealing with biological systems, it is not always meaningful to adhere to rigid criteria, and other issues have been considered, case-by-case, in reaching conclusions regarding particular sets of data. These are identified in the relevant text.

### B.6.1 Absorption, distribution, excretion and metabolism (IIA 5.1)

Figure 6.1. Structure of methomyl (\* position of radiolabel)



Chemical name: S-methyl-N-[(methylcarbamoyl)oxy]-thioacetimidate.

Molecular weight: 162.

Physical state: White crystalline compound

Octanol/water partition coefficient:  $\log k_{ow}$  0.09

Methomyl was labelled in the 1-C position of the molecule to follow the potential metabolism of methomyl to acetamide (potential carcinogen). In addition,  $^{14}\text{C}$ -methomyl was mixed with  $^{13}\text{C}$ -methomyl to aid in the mass spectral interpretation of metabolites.

#### B.6.1.1 Absorption, distribution and excretion

Three company metabolism studies are summarised in the following sections (2 rat studies and 1 primate study). All studies were conducted with  $^{14}\text{C}$ -methomyl at a single dose level of 4-5 mg/kg bw. One study used male rats preconditioned with methomyl in the diet (200 ppm) for 7, 8, or 19 days prior to receiving 4-5 mg/kg bw  $^{14}\text{C}$ -methomyl. The company considered the use of higher dose levels to be not feasible due to the oral toxicity of methomyl (an acute oral LD50 value of 17-23 mg/kg bw is cited in Hawkins *et al*, 1991). A significantly lower dose was also considered not feasible due to the large amounts of volatile metabolites expired and the difficulty in quantifying various other excreted metabolites using a smaller amount of dosed radioactivity. Additional information on the metabolism of methomyl in rats has been published by Huhtanen and Dorough (*Pestic. Biochem and Phys.*, Vol. 6, p. 571-583, 1976) and provides information on the methomyl metabolic pathway to acetonitrile and carbon dioxide.

## B.6.1.1.1 Rat

a)

<b>Report</b>	Hawkins, D.R., Mayo, B.C., Pollard, A.D., Haynes, L.M. (1991). The metabolism of [ $^{14}\text{C}$ ] methomyl in rats. Unpublished DuPont Report No. AMR 1584-90 (in-life phase: 1990).
<b>Test facility</b>	Huntingdon Research Centre (UK), Huntingdon, Cambridgeshire, U.K.
<b>Guidelines</b>	USEPA 85-1 (1982).
<b>Deviations from guidelines</b>	None
<b>GLP</b>	Yes (certified by the UK regulatory authorities).
<b>14C-methomyl Batch No Radiochemical purity Specific activity</b>	C# 312 (Lot No 2449-040) >97% 66.9 $\mu\text{Ci}/\text{mg}$ (adjusted to 31.3 $\mu\text{Ci}/\text{mg}$ with 13C-methomyl)
<b>13C-methomyl Batch No Purity</b>	C#348 (Lot No 2565-151) 99.9
<b>Acceptable</b>	Yes

**Materials and methods**

Charles River Crl:CD BR rats (5/sex) were orally (gavage) administered 14C-methomyl and/or a mixture of 14C- and 13C-methomyl at a dose level of 5 mg/kg bw in a solution of 0.1 M sodium acetate buffer (pH 5). The nominal dose volume was 1 ml/kg. Each dosing solution contained enough radiolabel to deliver 26-30  $\mu\text{Ci}/\text{animal}$ . The radiochemical purity of the test substance dosing solution was greater than 97% with approximately 50% 13C-methomyl. A pilot study was carried out in two rats (1/sex) using a single oral dose of 14C-methomyl.

Immediately after dosing, each rat was placed in a separate glass metabolism chamber. Urine samples (cooled with dry ice) were collected at 0-6, 6-24 hours and at 24-hour intervals for 7 days. Faeces were collected at 24-hour intervals for 7 days. Effluent air from the metabolism cages was passed through the following traps for 5 days to collect volatile organic metabolites: methanol, 300 ml (ambient temperature), methanol, 300 ml (cooled in dry ice) and two traps containing ethanolamine:2-ethoxyethanol (1:4, v/v, 300 mL) to collect  $^{14}\text{CO}_2$ . The rats were sacrificed 7 days after dosing by exsanguination under halothane anaesthetic by cardiac puncture. Blood samples were taken. The following organs and tissues were taken for analysis: heart, lungs liver spleen, gastrointestinal tract brain, ovaries testes, total skin, and samples of muscle, fat and bone. Kidneys were sampled, but lost prior to analysis. The remaining carcass was retained for measurement. At sacrifice, the cage was thoroughly washed with dilute detergent, water, and acetone; and these were pooled before measurement. Samples were stored at  $-20^\circ\text{C}$  until analysis. Radioactivity in all samples was determined using liquid scintillation counting (LSC) techniques.

**Results**

Following oral administration (5 mg/kg bw methomyl), both male and female rats exhibited clinical signs typical of acetylcholinesterase inhibition such as muscle tremors and humped posture. Onset of clinical signs was observed within 15 minutes

of dosing and subsided within 2 hours after dosing. No deaths or other abnormalities were noted apart from the rats being lethargic during the 2-hour post treatment period.

The radioactivity levels in expired air, urine, faeces and cage wash are presented in Table 6.1.

Table 6.1 Excretion of radioactivity after a single oral dose of  $^{14}\text{C}$ -methomyl (5 mg/kg bw)

Sample		Percent of dose (Mean $\pm$ SD)	
		Males (n = 5)	Females (n = 5)
<b>Expired air</b>			
<b><math>^{14}\text{C}</math>-acetonitrile</b>			
Hours	0-6	3.36 $\pm$ 0.81	3.47 $\pm$ 0.36
	6-24	6.63 $\pm$ 1.93	6.66 $\pm$ 0.93
	24-48	1.51 $\pm$ 0.45	1.95 $\pm$ 0.23
	48-72	0.35 $\pm$ 0.09	0.45 $\pm$ 0.08
	72-96	0.14 $\pm$ 0.03	0.18 $\pm$ 0.03
	96-120	0.11 $\pm$ 0.02	0.14 $\pm$ 0.03
<sup>a</sup> Total		12.09 $\pm$ 3.26	12.85 $\pm$ 1.41
<b><math>^{14}\text{CO}_2</math></b>			
Hours	0-6	17.42 $\pm$ 0.78	18.11 $\pm$ 0.94
	6-24	2.51 $\pm$ 0.75	2.91 $\pm$ 0.13
	24-48	0.79 $\pm$ 0.21	0.88 $\pm$ 0.12
	48-72	0.50 $\pm$ 0.05	0.51 $\pm$ 0.05
	72-96	0.32 $\pm$ 0.11	0.36 $\pm$ 0.03
	96-120	0.29 $\pm$ 0.09	0.36 $\pm$ 0.07
<sup>a</sup> Total		21.83 $\pm$ 1.40	23.13 $\pm$ 1.01
<b>Urine</b>			
Hours	0-6	45.61 $\pm$ 7.77	45.36 $\pm$ 4.59
	6-24	3.88 $\pm$ 0.31	4.01 $\pm$ 0.21
	24-48	1.31 $\pm$ 0.40	1.14 $\pm$ 0.22
	48-72	0.73 $\pm$ 0.17	0.72 $\pm$ 0.14
	72-96	0.50 $\pm$ 0.13	0.48 $\pm$ 0.06
	96-120	0.40 $\pm$ 0.05	0.44 $\pm$ 0.06
	120-144	0.25 $\pm$ 0.06	0.30 $\pm$ 0.07
	144-168	0.27 $\pm$ 0.06	0.29 $\pm$ 0.06
Total		52.94 $\pm$ 6.77	52.73 $\pm$ 4.16
Cage wash		0.16 $\pm$ 0.07	0.13 $\pm$ 0.04
<b>Faeces</b>			
Hours	0-24	0.99 $\pm$ 0.97	0.66 $\pm$ 0.33
	24-48	0.38 $\pm$ 0.09	0.39 $\pm$ 0.16
	48-72	0.40 $\pm$ 0.23	0.37 $\pm$ 0.07
	72-96	0.21 $\pm$ 0.05	0.21 $\pm$ 0.06
	96-120	0.59 $\pm$ 0.73	0.17 $\pm$ 0.04
	120-144	0.15 $\pm$ 0.07	0.12 $\pm$ 0.04
	144-168	0.14 $\pm$ 0.04	0.15 $\pm$ 0.05
Total		2.86 $\pm$ 0.07	2.07 $\pm$ 0.31

Key: a) Totals are a mean of individual totals and not the sum of the means.

The excretion pattern was the same between the pilot and the definitive study. There were no sex differences in the elimination rates. Absorption appeared to be almost complete as only 2-3% was found in the faeces. There were no statistical differences ( $p > 0.05$ ) in the excretion rate between the sexes. The estimated half-life was 5 hours with most of the absorbed dose (approximately 80%) eliminated in the first 24 hours

after dosing including 49% in the urine, 1% in faeces 20% as expired  $^{14}\text{CO}_2$  and 10% as expired  $^{14}\text{C}$ -acetonitrile.

The mean amounts of radioactivity in the tissues at sacrifice are presented in Table 6.2.

Table 6.2 Mean radioactive residues ( $\mu\text{g equiv/g}$  and % of dose) in tissues samples from rats sacrificed at 168 hours after dosing

Sample	Dose 5 mg/kg bw (gavage)			
	Males		Females	
	$\mu\text{g equiv/g} \pm \text{SD}$	% dose $\pm \text{SD}$	$\mu\text{g equiv/g} \pm \text{SD}$	% dose $\pm \text{SD}$
Bone	$0.18 \pm 0.05$	$0.1 \pm <0.01$	$0.24 \pm 0.04$	$0.1 \pm 0.01$
Spleen	$0.39 \pm 0.04$	$0.02 \pm <0.04$	$0.57 \pm 0.07$	$0.03 \pm 0.01$
Liver	$0.35 \pm 0.05$	$0.46 \pm 0.05$	$0.36 \pm 0.06$	$0.37 \pm 0.07$
Blood cells	$3.12 \pm 0.58$	$1.54 \pm 0.29$	$3.87 \pm 0.85$	$1.91 \pm 0.42$
Fat	$0.20 \pm 0.04$	$0.02 \pm 0.01$	$0.18 \pm 0.06$	$0.02 \pm 0.01$
Muscle	$0.14 \pm 0.01$	$0.06 \pm <0.01$	$0.14 \pm 0.01$	$0.05 \pm <0.01$
Lungs	$0.38 \pm 0.04$	$0.04 \pm 0.01$	$0.74 \pm 0.17$	$0.09 \pm 0.02$
Brain	$0.26 \pm 0.02$	$0.05 \pm 0.01$	$0.25 \pm 0.04$	$0.05 \pm 0.01$
Heart	$0.44 \pm 0.14$	$0.05 \pm 0.01$	$0.55 \pm 0.09$	$0.05 \pm 0.01$
Testes	$0.18 \pm 0.02$	$0.05 \pm 0.01$	-	-
Ovaries	-	-	$0.33 \pm 0.07$	$<0.01$
Gastrointestinal tract	$0.28 \pm 0.10$	$0.65 \pm 0.20$	$0.27 \pm 0.03$	$0.53 \pm 0.23$
Skin	$0.43 \pm 0.20$	$2.48 \pm 0.99$	$0.47 \pm 0.07$	$0.47 \pm 0.07$
Plasma	$0.68 \pm 0.30$	$0.37 \pm 0.16$	$0.93 \pm 0.43$	$0.50 \pm 0.04$
Carcass	$1.85 \pm 0.35$	$2.16 \pm 0.32$	$2.34 \pm 0.59$	$2.65 \pm 0.59$

At sacrifice (168 hours after dosing), some radioactivity was retained in the gastrointestinal tract (0.6%), skin, (2.5%), whole blood (3%), and liver (0.4%). The concentrations of radioactivity were highest in blood cells (3-4  $\mu\text{g equiv/g}$ ) and in plasma (0.7-0.9  $\mu\text{g equiv/g}$ ). Assuming a plasma cell volume of 48%, whole blood concentrations were calculated to be 1.9-2.3  $\mu\text{g equiv/g}$ . The concentrations of radioactivity in all other tissues were lower than whole blood or in blood cells or plasma alone. Tissues and organs highly perfused with blood (lung spleen, heart, liver) generally had higher concentrations (0.4 to 0.7  $\mu\text{g equiv/g}$ ) than organs less perfused such as gonads or bone, fat (0.1-0.3  $\mu\text{g equiv/g}$ ). Kidney samples were lost prior to analysis; however, previous rat metabolism studies conducted with  $^{14}\text{C}$ -methomyl (cross index, Harvey *et al.*, 1971) have shown that kidney residues were lower than blood residues and comparable to liver residues. The tissue residues accounted for approximately 8% and 9% of the administered radioactivity in males and females, respectively. There were no appreciable differences in the concentrations in tissues between male and female rats.

The overall material balance is presented in Table 6.3.

Table 6.3 Mean disposition and material balance from rats dosed with <sup>14</sup>C-methomyl

No of rats	Sex	Urine & cage wash	Faeces	Expired <sup>14</sup> C-CO <sub>2</sub>	Expired <sup>14</sup> C-acetonitrile	Total carcass	Total recovered
<b>Pilot study</b>							
1	M	54.71	1.78	15.81	13.07	9.72	95.09
1	F	59.83	1.48	21.55	12.35	10.07	105.28
<b>Main study</b>							
5	M	53.11 ± 6.72	2.86 ± 1.23	21.83 ± 1.40	12.09 ± 3.26	7.88	97.78
5	F	52.86 ± 4.16	2.07 ± 0.31	23.13 ± 1.01	12.85 ± 1.41	8.62	99.54

## Conclusions

Approximately 53% of the methomyl dose was excreted in the urine, 22% as respiratory <sup>14</sup>CO<sub>2</sub>, 13% as respiratory <sup>14</sup>C-acetonitrile, and 2-3% in the faeces. The excretion half-lives for total radioactivity were similar for male and female rats and estimated to be about 5 hours. Absorption was rapid and appeared to be almost complete (only 2-3% was found in the faeces). The total radioactive tissue levels in all animals were lower or similar to plasma levels, with the exception of red blood cells, indicating no specific bioaccumulation in tissues. Between 8 and 9% of the dose was in the tissues and carcass at 168 hours. The overall recovery was acceptable (95-105%) for both the pilot and main study.

(Hawkins *et al*, 1991)

b)

<b>Reports</b>	a) Harvey, J. Jr., Jelinek, A.G., Sherman, H. (1967). Metabolism of S-methyl N-[(methylcarbamoyloxy]thioacetimidate in the rat. Unpublished DuPont Report No.: ML/ME 24 (in-life phase: not specified). b) Harvey, J. Jr., Buchanan, J.B. (1968). Absence of S-oxide and S,S dioxide as potential metabolites of methomyl in soil, tobacco and rats. Unpublished DuPont Report No. ML/ME 25. c) Harvey, J. Jr., Jelinek, A.G., Sherman, H. (1973). Metabolism of methomyl in the rat. Published DuPont Report No. ML/ME 50 ( <i>J. Agric. Food Chem.</i> No. 5, p 769-775, 1973). This published paper is based on the two unpublished company studies and provides additional information on materials and methods.
<b>Test facility</b>	DuPont Experimental Station, Wilmington, DE.
<b>Guidelines</b>	None
<b>Deviations from guidelines</b>	Not applicable
<b>GLP</b>	No
<b><sup>14</sup>C-methomyl Batch No</b>	No details in the reports.
<b>Radiochemical purity</b>	>99.9%.
<b>Specific activity</b>	6.08 µCi.
<b>Methomyl Batch No</b>	Technical grade
<b>Purity</b>	No details in the reports.
<b>Acceptable</b>	Not acceptable for regulatory purposes on a stand alone basis (but contains relevant scientific information).

## Materials and methods

Three male Charles River Crl:CD rats were administered diet containing 200 ppm technical grade methomyl for 7, 8, or 19 days. After preconditioning, each rat was administered a single oral dose (gavage) of approximately 4-5 mg mg/kg bw <sup>14</sup>C-S-

methyl-N-[(methylcarbamoyl)oxy]-thioacetimidate in 2 ml of peanut oil (6-7  $\mu\text{Ci}$  per rat). Immediately after dosing, each rat was placed in a separate glass metabolism chamber. Urine and faeces were collected separately over 24-hour intervals.

For two of the animals (preconditioned for 7 or 8 days), effluent air from the metabolism cages was passed through a sodium hydroxide trap for the collection of  $^{14}\text{CO}_2$ . For one animal (preconditioned 19 days), the effluent air was passed through a sodium hydroxide trap, then an oxidising furnace filled with cupric oxide heated at  $700^\circ\text{C}$  followed by another sodium hydroxide trap. Neutral organic compounds passing through the first trap were oxidised to  $^{14}\text{CO}_2$  and trapped in the last sodium hydroxide trap.

One animal was sacrificed at 24 hours (7 days preconditioning) and the other two at 72 hours. Blood samples were taken by cardiac puncture. Selected organs and tissues were taken for analysis (heart, lungs, liver, kidneys, spleen, gastrointestinal tract, brain, testes, total skin, samples of muscle and fat and the carcass). The samples were stored frozen until analysis.

Due to the discovery of a neutral volatile from the one animal, an additional experiment was conducted in which four rats were treated with  $^{14}\text{C}$ -methomyl as above and placed two at a time in glass metabolism cages (duration of preconditioning not stated). The effluent air was drawn immediately through two cold traps, the first cooled in dry ice and the other in liquid nitrogen. Aliquots of the liquid nitrogen trap were analysed by GC/MS.

Radioactivity in all samples was determined using LSC techniques. Small tissue samples were combusted *in toto* while larger organs were homogenised.

Urine was analysed directly by counter current exchange using the 100-tube benzene/water system in an E-C Apparatus Company model 520 counter current fractionator. Metabolites were also analysed by normal phase TLC. A polar urine fraction was also incubated with glucuronidase/sulphatase enzymes to determine if conjugates were present.

## Results

Following oral administration of the test substance (approximately 5 mg/kg bw  $^{14}\text{C}$ -methomyl) the rats exhibited clinical signs of acetylcholinesterase inhibition such as chewing motions, slight tremors, salivation, etc. Onset of clinical signs was observed within 11 minutes of dosing and subsided within 1 hour after dosing. No deaths or other abnormalities were noted.

Excretion data from the 3 experiments are presented as a percent of applied dose in Table 6.4. Male rats excreted between 16 and 27% of the administered dose in the urine, 1-2% in faeces, 15-23% as expired  $^{14}\text{CO}_2$ , and 33% as expired  $^{14}\text{C}$ -acetonitrile (measured from one animal). Approximately 9-10% of the dose remained in the tissues at 24 or 72 hours. Overall material balance was 52-66% of the administered dose.

Table 6.4. Mean disposition and material balance of radioactivity in rats preconditioned with 200 ppm methomyl in diet followed by approximately 5 mg/kg bw <sup>14</sup>C-methomyl by gavage (% of dose).

Days fed 200 ppm	Time of sacrifice (hours)	Urine	Faeces	Expired <sup>14</sup> CO <sub>2</sub>	Expired <sup>14</sup> C-acetonitrile	<sup>a</sup> Total tissues	Total recovery
7	24	27	<1	15	<sup>b</sup> NS	9	52
8	72	24	23	23	NS	10	60
19	72	16	17	17	33	NS	66

Key: a) All tissues and remaining carcass. b) NS = not sampled.

Radioactive components in urine were characterised by their behaviour during counter current fractionation. Virtually all the radioactivity was found as a very polar material in the first few lower phases. No more than 4% was found in fractions where methomyl would be located. This material was extracted into chloroform, however, when concentrated; it was lost as a volatile. This behaviour was consistent with acetonitrile (IN-07467), which would have appeared in the same fractions as methomyl. Less than 0.1% was found in fractions corresponding to methomyl oxime<sup>1</sup> or methomyl *S,S*-dioxide (IN-M1284). The polar material was isolated and treated with  $\beta$ -glucuronidase-aryl sulphatase. TLC analyses demonstrated that none of the radioactivity was associated as methomyl oxime or methomyl *S*-oxide (IN-W1602).

Radioactivity in cold traps used to collect organic volatiles in expired air was analysed directly by GC/MS and confirmed to be <sup>14</sup>C-acetonitrile (IN-07467). Radioactivity in the sodium hydroxide traps was precipitated as the barium salt of <sup>14</sup>C-carbonate by the addition of barium chloride confirming it was <sup>14</sup>CO<sub>2</sub>.

The amounts of radioactivity reported in the tissues are summarised in Table 6.5. In general, similar amounts of radioactivity were found in the two rats at the different sacrifice times. In the animals sacrificed at 24 and 72 hours, some radioactivity (as a % of dose) was retained in the gastro-intestinal tract (1.5-2.5%), skin (1.6-2.6%), whole blood (0.5-1.3%), and liver (0.4-0.6%). Total radioactivity retained in the tissues and carcass accounted for approximately 9-10% of the administered dose.

<sup>1</sup> methomyl oxime is also known by the codes MHTA and IN-X1177

Table 6.5. Radioactive residues in tissue samples from rats at 24 and 72 hours post dosing

Sample tissue	Rat sacrificed at 24 hours		Rat sacrificed at 72 hours	
	Total $\mu\text{Ci}$ in sample	<sup>a</sup> % dose	Total $\mu\text{Ci}$ in sample	<sup>a</sup> % dose
Spleen	0.003	0.05	0.003	0.05
Liver	0.042	0.65	0.035	0.54
Blood	0.034	0.52	0.084	1.29
Fat	0.002	0.03	>0.000	0.00
Muscle	0.004	0.06	0.003	0.05
Lungs	0.005	0.08	0.004	0.06
Brain	0.003	0.05	0.003	0.05
Heart	0.003	0.05	0.005	0.08
Testes	0.006	0.09	0.005	0.08
Kidneys	0.010	0.15	0.007	0.11
Gastrointestinal tract	0.167	2.57	0.122	1.88
Skin	0.105	1.62	0.169	2.60
Carcass	0.227	3.49	0.234	3.60
Total	0.611	9.40	0.674	10.37

## Conclusions

The radioactivity is rapidly eliminated in the ratio of one part  $^{14}\text{CO}_2$ , two parts  $^{14}\text{C}$ -acetonitrile (IN-07467) and one part urinary metabolites following 7-19 day dietary preconditioning with 200 ppm and a single dose of  $^{14}\text{C}$ -methomyl. The chemical identity of the radiolabelled material excreted in the urine was not established in this early work. No methomyl, methomyl *S*-oxide (IN-W1602), methomyl *S,S*-dioxide (IN-M1284), or methomyl oxime were detected in the urine which consisted mainly of a polar fraction.

(Harvey *et al*, 1967, Harvey and Buchanan, 1968 & Harvey *et al*, 1973)

### B.6.1.1.2 Monkey

<b>Report</b>	Hawkins, D.R., Mayo, B.C., Pollard, A.D., Haynes, L.M. (1992). The metabolism of $^{14}\text{C}$ -methomyl in male Cynomolgus monkey. Unpublished DuPont Report No. AMR 1902-90 [in-life phase: 29 July 1991 to 30 March 1991 (serial dosing of monkeys)].
<b>Test facility</b>	Huntingdon Research Centre (UK), Huntingdon, Cambridgeshire, U.K.
<b>Guidelines</b>	USEPA, Subdivision F, 85-1 (1982)
<b>Deviations from guidelines</b>	None
<b>GLP</b>	Yes (certified by the UK regulatory authorities).
<b><math>^{14}\text{C}</math>-methomyl Batch No Radiochemical purity Specific activity</b>	C#380 (Lot No 2729-122) >97% 44.8 $\mu\text{Ci}/\text{mg}$ (adjusted to 15.1 $\mu\text{Ci}/\text{mg}$ with $^{13}\text{C}$ -methomyl and unlabelled methomyl).
<b><math>^{13}\text{C}</math>-methomyl Batch No Purity</b>	C#348 (Lot No 2565-151) 99.9%
<b>Methomyl Batch No Purity</b>	DPX-X1179-379 98.9%
<b>Acceptable</b>	Yes



## Materials and methods

Four male Cynomolgus monkeys (*Macaca fascicularis*) aged 1-2.5 year old were administered a single oral (gavage) dose of methomyl using a mixture of  $^{14}\text{C}$ - and  $^{13}\text{C}$ -methomyl at a dose of 5 mg/kg bw. Each dose solution contained enough radiolabel to deliver 154 to 175  $\mu\text{Ci}$  per animal using 0.1 M sodium acetate buffer (pH 5) as the dose vehicle. The nominal dose volume was 4 ml/kg bw. The radiochemical purity of the test substance dosing solution was greater than 97% and contained approximately 50%  $^{13}\text{C}$ -methomyl. Dose selection was based on signs of acetylcholinesterase inhibition (mild miosis) at 5 mg/kg bw in a previous primate study (Hazleton Laboratories Report No 201-219, 1968).

Immediately after dosing, each monkey was placed in a separate stainless steel metabolism cage. The steel cage was housed in a sealed 1.0 m<sup>3</sup> chamber designed for the collection of expired air. Effluent air from the metabolism cages was passed through the following traps to collect volatile organic metabolites: methanol, 3.5 l (ambient temperature), methanol, 3.5 l (cooled in dry ice), an empty cold trap (-70°C, for 3 of the animals), and two traps containing 10% potassium hydroxide (15-30 l) to collect  $^{14}\text{CO}_2$ .

Urine was collected separately in containers cooled in dry ice at 6 and 24 hours, and then daily for 7 days. Faeces and cage debris were collected separately for each animal at 24-hour intervals for 7 days.

At 168 hours, monkeys were sedated with ketamine hydrochloride, and a blood sample was taken using heparinised syringes. Monkeys were then sacrificed using pentobarbitone followed by exsanguination. The blood sample was used for obtaining plasma and blood cells. The following organs and tissues were taken for analysis: heart, lungs, kidneys, liver, spleen, gastrointestinal tract, brain, testes, total skin and samples of muscle, fat and bone. The remaining carcass was retained for analysis. At sacrifice the cage was thoroughly washed with dilute detergent, water, and acetone; and these were pooled before measurement. Samples were stored at -15°C until analysis.

Radioactivity in all samples was determined using LSC techniques. Urine was analysed directly by HPLC using a Hamilton PRP-1 reversed phase column. Specific analyses for acetamide were conducted by HPLC using an Aminex HPXH Ion Partition column. Metabolites were also analysed by normal phase TLC. Various urine samples were also incubated with glucuronidase/sulphatase enzymes and analysed by TLC or HPLC to determine if conjugates were present.

## Results

Following oral administration of the test substance, no clinical signs of acetylcholinesterase inhibition were observed (but cholinesterase levels were not determined in his study). No deaths or other abnormalities were noted apart from one monkey having a rectal prolapse 4 hours after dosing. This was attributed to be a reaction to stress due to handling during dosing. The excretion data are presented in Table 6.6.

Table 6.6 Excretion of radioactivity (% of dose) after a single oral dose of  $^{14}\text{C}$ -methomyl (5 mg/kg bw)

Sample intervals	Animal Number				Mean ± SD
	1	2	3	4	
Expired air					
<b><sup>14</sup>C-acetonitrile</b>					
Hours 0-6	1.26	1.45	151	2.29	1.63 ± 0.45
6-24	1.44	2.52	2.66	2.94	2.39 ± 0.66
24-48	0.86	1.04	1.99	1.57	1.37 ± 0.51
<sup>a</sup> Total	3.56	5.01	6.16	6.80	5.38 ± 1.42
<b><sup>14</sup>CO<sub>2</sub></b>					
Hours 0-6	16.12	15.64	19.69	13.99	16.36 ± 2.40
6-24	14.55	15.54	9.99	22.34	15.61 ± 5.10
24-48	1.63	1.57	1.49	1.90	1.65 ± 0.18
<sup>a</sup> Total	32.30	32.75	31.17	38.23	33.61 ± 3.15
Total expired air	35.86	37.76	37.33	45.03	39.00 ± 4.10
Urine					
Hours 0-6	14.16	22.67	23.99	10.44	17.82 ± 6.57
6-24	7.78	8.47	8.15	10.89	8.82 ± 1.41
24-48	1.34	1.16	1.65	1.33	1.37 ± 0.20
48-72	0.34	0.34	0.35	0.32	0.34 ± 0.01
72-96	0.15	0.25	0.25	0.20	0.21 ± 0.05
96-120	0.12	0.14	0.20	0.11	0.14 ± 0.04
120-144	0.08	0.11	0.15	0.10	0.11 ± 0.03
144-168	0.06	0.11	0.11	0.07	0.09 ± 0.03
<sup>a</sup> Total	24.03	33.25	34.85	23.46	28.90 ± 5.99
Cage wash	5.98	2.29	1.26	3.81	3.34 ± 2.05
Total urine and cage wash	30.01	35.54	36.11	27.27	32.23 ± 4.30
Faeces					
Hours 0-24	0.55	0.38	0.23	0.38	0.39 ± 0.13
24-48	2.22	2.90	1.80	2.17	2.27 ± 0.46
48-72	0.26	0.15	0.24	0.35	0.25 ± 0.08
72-96	0.07	0.08	0.18	0.13	0.12 ± 0.05
96-120	0.09	0.05	0.07	0.04	0.06 ± 0.02
120-144	0.05	0.06	0.04	0.06	0.05 ± 0.01
144-168	0.04	0.02	0.03	0.03	0.03 ± 0.01
<sup>a</sup> Total	3.28	3.64	2.59	3.16	3.17 ± 0.44
Cage debris	0.39	0.15	0.44	0.27	0.31 ± 0.13

Key: a) Totals are a mean of individual totals and not the sum of the means.

The animals excreted a total of  $32.2 \pm 4.3\%$  of the radioactivity in the urine and  $3.2 \pm 0.4\%$  in faeces, with  $33.6 \pm 3.2\%$  and  $5.4 \pm 1.4\%$  in expired air as  $^{14}\text{CO}_2$  and  $^{14}\text{C}$ -acetonitrile, respectively. More than 62% of the dose was eliminated within 24 hours. The estimated half-life was 20 hours.

The mean amounts of radioactivity in the tissues at sacrifice are presented in Table 6.7.

Table 6.7. Mean radioactive residues ( $\mu\text{g equiv/g}$  and % of dose) in tissues samples from monkeys sacrificed at 168 hours after dosing (n = 4)

Tissue sample	$\mu\text{g equiv/g} \pm \text{SD}$	Dose $\pm \text{SD}$
Brain	$0.16 \pm 0.01$	$0.09 \pm 0.01$
Bone <sup>a</sup>	$0.06 \pm 0.01$	$0.02 \pm <0.01$
Bone marrow <sup>b</sup>	$0.04 \pm 0.09$	$0.03 \pm <0.01$
Fat <sup>b</sup>	$0.59 \pm 0.16$	$1.62 \pm 0.46$
Gastrointestinal tract	$0.21 \pm 0.04$	$0.35 \pm 0.03$
Heart	$0.21 \pm 0.02$	$0.01 \pm <0.01$
Kidneys	$0.45 \pm 0.05$	$0.04 \pm <0.01$
Liver	$0.82 \pm 0.12$	$0.34 \pm 0.03$
Lungs	$0.26 \pm 0.02$	$0.03 \pm <0.01$
Muscle <sup>b</sup>	$0.18 \pm <0.01$	$1.35 \pm 0.08$
Skin <sup>b</sup>	$0.34 \pm 0.06$	$0.59 \pm 0.10$
Spleen	$0.31 \pm 0.02$	$0.01 \pm 0.00$
Testes	$0.26 \pm 0.09$	$0.01 \pm -$
Whole blood <sup>b</sup>	$0.15 \pm 0.01$	$0.27 \pm 0.03$
Total	-	$4.76 \pm 0.58$

Key: a) Calculated using the weight of both femurs. b) Calculated assuming that these account for 0.35% (bone marrow), 14% (fat), 39% (muscle), 9% (whole blood) and 9% of the body weight.

Concentrations of radioactivity were highest in liver, fat, and kidneys (0.8, 0.6, and 0.5  $\mu\text{g equiv/g}$ , respectively). Intermediate concentrations of radioactivity were found in other tissues (0.1-0.5  $\mu\text{g/equiv/g}$ ) and were generally higher than concentrations in the whole blood (0.2  $\text{g/equiv/g}$ ). At 168 hours after dosing, approximately 5% of the radioactivity was retained in the tissues, mostly in fat (1.6%) and muscle (1.4%).

Table 6.8 Mean disposition and material balance from monkeys dosed with  $^{14}\text{C}$ -methomyl

Animal number	Urine and cage wash	Faeces	Cage debris	Expired $^{14}\text{CO}_2$	Expired $^{14}\text{C}$ -acetonitrile	Tissues	Total recovery
1	30.1	3.28	0.39	32.30	3.56	4.63	74.26
2	35.54	3.64	0.15	32.75	5.01	4.98	82.07
3	36.11	2.59	0.44	31.17	6.16	4.02	80.49
4	27.27	3.16	0.27	38.23	6.80	5.40	81.13
Mean $\pm$ SD	$32.23 \pm 4.30$	$3.17 \pm 0.44$	$0.31 \pm 0.13$	$33.61 \pm 3.15$	$5.38 \pm 1.42$	$4.76 \pm 0.58$	$79.47 \pm 3.60$

Key: a) Expired air monitored for 48. b) Excreta collected for 168 hours.

## Conclusions

Approximately 32% of the radioactivity was excreted in the urine, 3-4% in the faeces with 34% and 5% expired as  $^{14}\text{CO}_2$  and  $^{14}\text{C}$ -acetonitrile, respectively. About 5% remained in the tissues at 168 hours. The overall material balance was approximately 80-82% of the administered dose in three animals and 74% in one animal. The volatiles in expired air were not collected after 48 hours in order not to unduly stress the monkeys in the latter part of the study. This may have been a factor in not obtaining 100% material balance but it probably wouldn't account for 20%.

(Hawkins *et al*, 1992)

### B.6.1.2. Metabolism

The codes, structures and nomenclature for the metabolites are presented in Appendix 3. The proposed metabolic pathways for the rat and monkey are presented in Figure 6.2 and 6.3, respectively.

#### B.6.1.2.1 Rat

a)

<b>Report</b>	Hawkins, D.R., Mayo, B.C., Pollard, A.D., Haynes, L.M. (1991). The metabolism of [1- <sup>14</sup> C]methomyl in rats. Unpublished DuPont Report No. AMR 1584-90 (in-life phase: 1990).
<b>Test facility</b>	Huntingdon Research Centre (UK), Huntingdon, Cambridgeshire, U.K.
<b>Guidelines</b>	USEPA 85-1 (1982).
<b>Deviations from guidelines</b>	None
<b>GLP</b>	Yes (certified by the UK regulatory authorities).
<b>14C-methomyl Batch No</b>	C# 312 (Lot No 2449-040)
<b>Radiochemical purity</b>	>97%
<b>Specific activity</b>	66.9 µCi/mg (adjusted to 31.3 µCi/mg with 13C-methomyl)
<b>13C-methomyl Batch No</b>	C#348 (Lot No 2565-151)
<b>Purity</b>	99.9
<b>Acceptable</b>	Yes

### Materials and methods

Details of the dosing procedures and in-life phase are presented in section B.6.1.1.1a.

Metabolites were analysed by HPLC and normal phase TLC. Urine was analysed directly by HPLC using a Hamilton PRP-1 reversed phase column and LSC. Specific analyses for acetamide were conducted by HPLC using an Aminex HPXH Ion Partition HPLC column. Specific analyses for thiocyanate in the urine (pilot study only) were conducted with an OmniPac PAX-500 ion exchange column. The major urine metabolite was isolated by HPLC and analysed by liquid chromatography/mass spectrometry (fast atom bombardment MS) and by proton and carbon-13 NMR. Various urine samples were also incubated with glucuronidase/sulphatase enzymes to determine if conjugates were present.

### Results

The mean amounts of radioactive components measured in 0 to 24 hours urine are summarised in Table 6.9. No methomyl or methomyl oxime were found in urine. One major metabolite (17-18% methomyl mercapurate) and more than 10 minor metabolites were found in urine. Minor metabolite fractions generally ranged from 1 to 7% of the dose and most appeared to consist of more than one metabolite.

Table 6.9. Mean radioactive components (% dose) in 0-24 hour urine samples from male and female rats

<sup>a</sup> Metabolite fraction	Metabolites found	Male	Female
1	Polar components Acetamide	2.2 ± 0.6 0.4 ± 0.3	2.1 ± 0.6 0.2 ± 0.1
2		2.4 ± 0.6	2.4 ± 0.4
3		2.0 ± 0.6	2.7 ± 0.7
4	3-4% MHTA-sulphate (IN-CVA19) & 1-2% & acetic acid	5.0 ± 1.1	5.4 ± 0.7
5	Acetonitrile (~2%), other (3%)	5.7 ± 0.5	5.8 ± 0.5
6		2.6 ± 0.3	3.1 ± 0.5
7	Methomyl mercapurate (IN-KA129)	16.5 ± 3.6	17.6 ± 2.1
8	Several minor metabolites	5.7 ± 1.1	3.3 ± 0.3
9		0.1 ± 0.1	0.3 ± 0.1
Other	Minor peaks between fractions	6.9 ± 0.8	6.3 ± 0.5

Key: a) Radioactive components were characterised by their retention time as fractions eluting from the reversed phase HPLC column. b) Numerous minor metabolites could not be resolved completely; some fractions are the aggregate total of multiple minor metabolites.

Metabolite fraction 5 contained approximately 2% <sup>14</sup>C-acetonitrile (IN-07467) and approximately 3% of other non-volatile components. Metabolite fraction 4 contained approximately 1-2% <sup>14</sup>C-acetic acid and 3-4 % MHTA-sulphate (IN-CVA19). Acetamide (IN-09066) was detected in fraction 1 and accounted for 0.2 to 0.4% of the dose. TLC analyses demonstrated that none of the radioactivity was associated as methomyl oxime or urea. Methomyl and the anti-isomers of methomyl (IN-B1884) and anti-isomer MHTA (IN-B1871) were not present in the urine. Urine profiles were not significantly changed after incubation with  $\beta$ -glucuronidase/sulphatase enzymes from three different sources. No aceohydroxamic acid (putative metabolite of acetamide) was detected in urine. After treatment with acid, most of the components in urine were converted to <sup>14</sup>C-acetate indicating that most of the urinary metabolites were composed of at least a 2 carbon fragment rather than being a one carbon fragment (e.g. <sup>14</sup>CO<sub>2</sub>) re-incorporated into larger components. Specific analyses for thiocyanate in the urine (pilot study only) revealed that this was a minor metabolite, which accounted for less than 2% of the dose.

Acetonitrile (IN-07467) was the major residue in blood cells, plasma, and liver accounting for 25-33% of the radioactivity in these tissues. Another major metabolite appeared to be a glucuronide of a polar compound. Most tissue residues were converted to acetate by acid hydrolysis, indicating a common two-carbon fragment. Acetonitrile (IN-07467) was the only significant radioactive organic volatile found in expired air together with <sup>14</sup>CO<sub>2</sub>.

An average of 2-3% of the dose was excreted in the faeces. Faeces samples were extracted with methanol/water in an attempt to characterise the radioactivity. Most radioactivity was unextractable under these conditions (less than 1% of the dose), precluding meaningful chromatographic analysis.

There is evidence for three major metabolic pathways. One pathway involves the displacement of the S-methyl moiety of methomyl with glutathione. The conjugate is

transformed *in vivo* to the corresponding mercapturic acid derivative by a series of enzymatic reactions. The mercapturic acid derivative (IN-KA129) was the major urinary metabolite of methomyl and accounted for approximately 18% of the administered dose.

Another major metabolic pathway involves cleavage of the carbamate ester to release methomyl oxime. This metabolite was not observed in the urine, indicating that it is rapidly metabolised or conjugated. Only a small amount of the sulphate conjugate of IN-X1177 (IN-CVA19) was tentatively identified as a urinary metabolite by chromatography. Experiments with glucuronidase did not release significant radioactivity corresponding to methomyl oxime, thus the majority of the released methomyl oxime was metabolised. Since  $^{14}\text{CO}_2$  has been shown to be a major degradate of methomyl oxime when orally administered to rats (Huhtanen and Dorough, 1976), it is likely to be the major precursor in the formation of  $^{14}\text{CO}_2$  which was 23% of the administered dose in this study.

The third proposed pathway involves *in vivo* isomerisation of methomyl to the anti-methomyl isomer, (IN-B1884) which was not detected in the urine. *Anti*-isomer of methomyl or the corresponding hydrolysis product IN-B1871 may undergo a Beckmann type rearrangement and elimination reaction to form acetonitrile (IN-07467), which is mostly eliminated in the expired air (approximately 12% of administered dose). The acetonitrile pathway is based on work conducted by Huhtanen and Dorough (1976) in which either the *syn*- or *anti*- form of methomyl was dosed to rats. The metabolism of  $^{14}\text{C}$ -methomyl (via acetonitrile) to  $^{14}\text{C}$ -acetamide (IN-09066) was a very minor pathway accounting for approximately 0.2-0.4% of dose. Thiocyanate was also a minor metabolite of acetonitrile, and accounted for less than 2% of the dose (data from pilot study).

## Conclusions

The metabolism of methomyl in the rat is extensive, rapid, and complex. The metabolite profiles in the urine were nearly identical between male and female rats. The major urine metabolite was the mercapturic acid derivative of methomyl (IN-KA129) accounting for about 18% of the dose. There were at least 10 other minor urinary metabolites. Acetonitrile was the major residue in blood and liver. Three major pathways were proposed: displacement of *S*-methyl from *syn*-methomyl by glutathione followed by transformation to the mercapturic acid derivative; conversion of methomyl to **methomyl oxime** and  $\text{CO}_2$  release; and isomerisation of *syn*-methomyl to *anti*-methomyl (IN-B1884), followed by a Beckmann rearrangement and formation of acetonitrile.

## B.6.1.2.2 Monkey

<b>Report</b>	Hawkins, D.R., Mayo, B.C., Pollard, A.D., Haynes, L.M. (1992). The metabolism of <sup>14</sup> C-methomyl in male Cynomolgus monkey. Unpublished DuPont Report No. AMR 1902-90 [in-life phase: 29 July 1991 to 30 March 1991(serial dosing of monkeys)].
<b>Test facility</b>	Huntingdon Research Centre (UK), Huntingdon, Cambridgeshire, U.K.
<b>Guidelines</b>	USEPA, Subdivision F, 85-1 (1982)
<b>Deviations from guidelines</b>	None
<b>GLP</b>	Yes (certified by the UK regulatory authorities).
<b><sup>14</sup>C-methomyl Batch No Radiochemical purity Specific activity</b>	C#380 (Lot No 2729-122) >97% 44.8 µCi/mg (adjusted to 15.1 µCi/mg with <sup>13</sup> C-methomyl and unlabelled methomyl).
<b><sup>13</sup>C-methomyl Batch No Purity</b>	C#348 (Lot No 2565-151) 99.9%
<b>Methomyl Batch No Purity</b>	DPX-X1179-379 98.9%
<b>Acceptable</b>	Yes

**Materials and methods**

Details of the dosing procedures and in-life phase are presented in section B.6.1.1.2.

Radioactive components were characterised by their retention time as fractions eluting from the reversed phase HPLC column. The separation and quantification of specific minor metabolites were carried out using various TLC systems and ion partition HPLC chromatography.

**Results**

The mean amounts of radioactive components measured in 0-24 hour urine are summarised in Table 6.10.

Table 6.10. Mean radioactive components (% dose) in 0-24 hour urine samples from male monkeys dosed with 5 mg/kg bw  $^{14}\text{C}$ -methomyl (n = 4)

Metabolite fraction	Metabolite found	Mean $\pm$ SD
1	Multiple components <sup>a</sup>	6.8 $\pm$ 0.9
	Acetamide	0.4 $\pm$ 0.1
2		1.9 $\pm$ 0.6
3		0.4 $\pm$ 0.2
4		0.3 $\pm$ 0.2
5	Acetic acid	0.4 $\pm$ 0.2
6	MHTA sulphate (IN-CVA19)	1.5 $\pm$ 1.0
		0.2
7	Acetonitrile <sup>b</sup>	1.7 $\pm$ 0.7
8		2.4 $\pm$ 0.7
9		2.2 $\pm$ 1.1
10		0.5 $\pm$ 0.1
11	Methomyl mercapturate (IN-KA129)	0.8 $\pm$ 0.4
12		2.5 $\pm$ 1.0
13		2.7 $\pm$ 1.0
other	Minor peaks between fractions	2.6 $\pm$ 1.0

Key: a) Sulphate/N-cysteine conjugate of acetonitrile was determined to be a component of Fraction 1.  
b) Acetonitrile was determined to be a major component of Fraction 7.

The radioactivity in the urine was composed of one major metabolite (fraction 1) and more than 10 other minor metabolites. Minor metabolite fractions generally ranged from 0.3 to 3% of the dose and most appeared to consist of more than one metabolite. Fraction 1 was found to consist of 7 or more components that included acetamide (IN-09066). None of these components was greater than 4% of the dose. A discrete radioactive component corresponding to MHTA-sulphate (IN-CVA19) was detected in the urine from only one animal and accounted for ~0.2% of the dose, in other animals this component was below the limit of detection. No radioactivity was associated with syn-or anti-MHTA (IN-X1177 and IN-B1871, respectively), methomyl *S*-oxide (IN-W1602), methomyl *S,S*-dioxide (IN-M1284), anti-methomyl (IN-B1884), MHTA *S*-oxide (IN-A3338), aceto-hydroxamic acid, or hydroxyl methyl methomyl (IN-G6520). Urine profiles were not significantly changed after incubation with  $\beta$ -glucuronidase/sulphatase. One of the polar metabolites in the urine was later identified by LC/MS and NMR as a sulfate/cysteine conjugate of acetonitrile (Reiser *et al.*, 1997, *J. Agric. Food Chem.*, Vol. 45, No. 6, pp. 2309-2313).

An average of 3-4% of the dose was excreted in the faeces. Approximately half of the radioactivity was extractable with methanol/water (~2% of the dose), but this small amount was not analysed further.  $^{14}\text{C}$ -acetonitrile (IN-07467) was the only significant radioactive organic volatile found in expired air together with  $^{14}\text{CO}_2$ .

Evidence for two major pathways exists, one resulting in the formation of  $^{14}\text{CO}_2$  and the other resulting in the formation of  $^{14}\text{C}$ -acetonitrile. In the  $^{14}\text{CO}_2$  pathway, the carbamate ester is cleaved, releasing methomyl oxime. Methomyl oxime was not observed in the urine, suggesting that it is rapidly metabolised or conjugated. Small amounts (0.2%) of the sulphate conjugate of the MHTA (IN-CVA19) was tentatively identified as a urinary metabolite from one animal. Incubation of the urine with  $\beta$ -glucuronidase did not release significant radioactivity corresponding to MHTA. Thus, the majority of the released methomyl oxime is metabolised. Since  $^{14}\text{CO}_2$  has



been shown to be a major degradate of methomyl oxime when orally administered to rats (Huhtanen and Dorrough, 1976), it is likely to be the major precursor in the formation of  $^{14}\text{CO}_2$ , which was 34% of administered dose in this study. In the acetonitrile pathway, methomyl isomerises to the anti-methomyl isomer, (IN-B1884) which was not detected in the urine. Anti-isomer of methomyl or the corresponding hydrolysis product anti-MHTA (IN-B1871) may undergo a Beckmann-type rearrangement and elimination reaction to form  $^{14}\text{C}$ -acetonitrile (IN-07467), which is mostly eliminated in the expired air (approximately 5% of administered dose).

The metabolism of  $^{14}\text{C}$ -methomyl (via acetonitrile) to  $^{14}\text{C}$ -acetamide (IN-09066) was a very minor pathway accounting for approximately 0.4% of dose. Other metabolic pathways resulting in the formation of numerous polar urinary metabolites were present. Minor metabolites tentatively identified included the mercapturic acid derivative of methomyl (IN-KA129), acetic acid, and a cysteine/sulphate conjugate of acetonitrile. Many of the unidentified metabolites (none of which represented more than 4% of the dose) had similar chromatographic behaviour to minor metabolites found in an earlier rat metabolism study (Hawkins *et al.*, 1991) and were the result of extensive metabolism of initial metabolites.

### Conclusions

Methomyl metabolite profiles in the urine were complex, with over 18 metabolites observed and none of which was greater than 4%. Two major pathways were proposed: hydrolysis of the carbamate ester of methomyl to methomyl oxime which is subsequently metabolised to  $\text{CO}_2$ ; and isomerisation of *syn*-methomyl to *anti*-methomyl, followed by a Beckmann rearrangement and formation of acetonitrile. A minor pathway involved displacement of *S*-methyl from *syn*-methomyl by glutathione followed by transformation to the mercapturic acid derivative (IN-KA129). Acetonitrile was further metabolised via conjugation with cysteine and sulphate.

(Hawkins *et al.*, 1992)

#### B.6.1.3 Dermal penetration

a) *In vivo*

Data are presented in section B.6.12.

b) *In vitro*

Data are presented in section B.6.12.

### B.6.1.4 Summary of mammalian metabolism

Methomyl was readily absorbed from the gastrointestinal tract (only 2-4% eliminated in faeces) and rapidly eliminated within 24 hours of dosing (80% in the rat and 63% in the monkey). Urinary excretion was the major route of elimination in rats whereas expired air was the major route in monkeys (Table 6.11). However, excretion via expired air was an important route of elimination in both the rat and monkey (approximately 30-35% of the radioactivity within 24 hours of dosing). Less than 4% of the dose was eliminated in the faeces. The excretion half-life is about 5 hours in the rat and between 12 and 24 hours in the monkey.

There were no differences in tissue residues between male or female rats. Total radioactive residues in the rat tissues were lower than in the plasma with the exception of red blood cells. Approximately 8-9% of the dose was retained in the rat at 168 hours and approximately 5% in the monkey. Following repeated exposure, approximately 9-10% of the administered dose was retained in the rat at 72-hours post treatment. There were no sex differences in the absorption, the rate of elimination or in the distribution and concentration of the tissue residues in rats.

Table 6.11 Summary of the elimination of radiolabelled methomyl in rats (both sexes combined) and monkeys dosed with 5 mg/kg bw/day 14-methomyl (approximate percent of the dose)

Parameter	0-24 hours	0-48 hours	0-168 hours
<b>Rats</b>			
14C-acetonitrile	10	12	12.5
14CO <sub>2</sub>	20	21	22
Urine	50	51	53
Faeces	0.8	1.2	2-3
Tissue residues	-	-	8-9 (at 168 hours)
<b>Monkeys</b>			
14C-acetonitrile	4	5	-
14CO <sub>2</sub>	32	34	-
Urine	27	28	29
Faeces	0.4	3	3
Tissue residues	-	-	5 (at 168 hours)

Key: a) Expired air collected for 48 hours only.

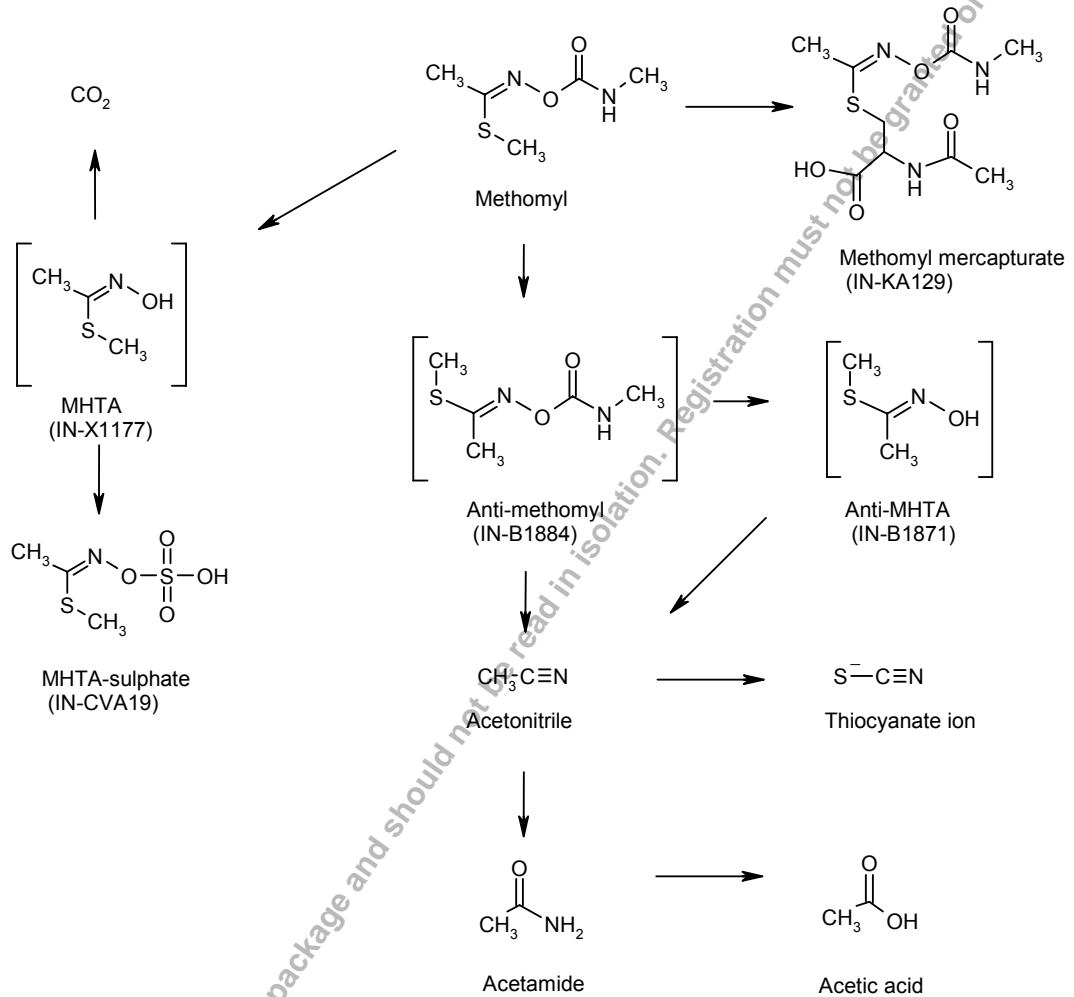
Metabolism was extensive in both the rat and monkey. In the rat, the metabolite profiles in the urine were nearly identical between male and female rats. The major urine metabolite was the mercapturic acid derivative of methomyl (IN-KA129) together with at least 10 other minor urinary metabolites. Acetonitrile was the major residue in blood and liver. Three major pathways were proposed: displacement of *S*-methyl from *syn*-methomyl by glutathione followed by transformation to the mercapturic acid derivative (18% of the dose); conversion of methomyl to methomyl oxime and CO<sub>2</sub> release; and isomerisation of *syn*-methomyl to *anti*-methomyl (IN-B1884), followed by a Beckmann rearrangement and formation of acetonitrile.

In the monkey, the metabolite profiles in the urine were complex, with over 18 metabolites observed and none of which was greater than 4%. Unmetabolised methomyl was not found in urine and less than 4% of the dose was eliminated in the faeces. Two major pathways were proposed: hydrolysis of the carbamate ester of methomyl to methomyl oxime which is subsequently metabolised to CO<sub>2</sub>; and isomerisation of *syn*-methomyl to *anti*-methomyl, followed by a Beckmann rearrangement and formation of acetonitrile. A minor pathway involved displacement of *S*-methyl from *syn*-methomyl by glutathione followed by transformation to the mercapturic acid derivative (IN-KA129). Acetonitrile was further metabolised via conjugation with cysteine and sulphate.

Although the metabolic pathway of methomyl in the monkey is similar to that of the rat, the monkey appears to excrete noticeably more <sup>14</sup>CO<sub>2</sub> and less <sup>14</sup>C-acetonitrile than the rat in expired air, the monkey excretes considerably less of the mercapturic acid derivative of methomyl in urine (0.8% v18%) and the monkey excreted a greater number of urinary metabolites.

WARNING: This document forms part of an EC evaluation data package and should not be read in isolation. Registrants must not be granted access to the body of this document.

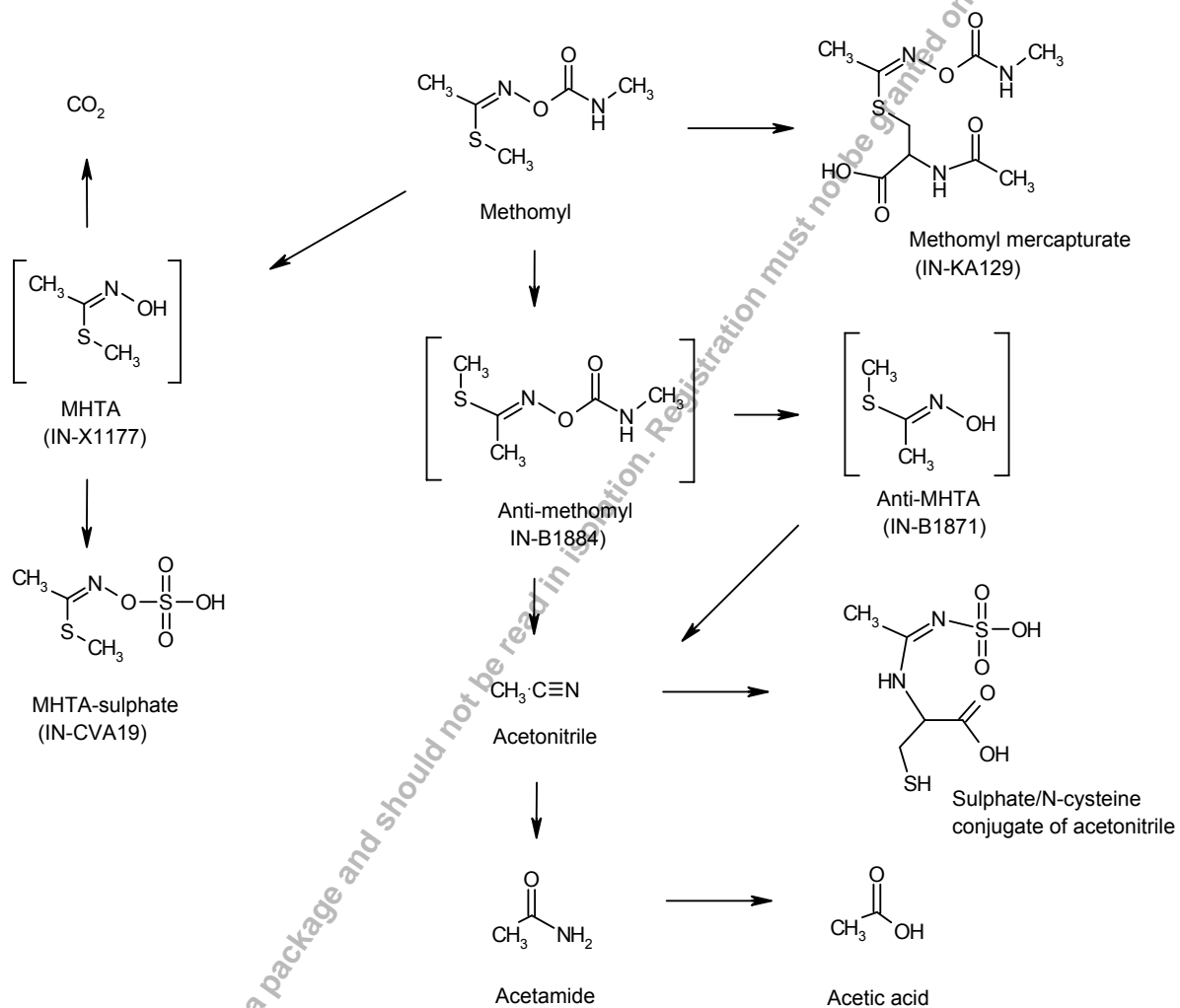
Figure 6.2

Proposed metabolic pathway of methomyl in the rat

[ ] proposed

WARNING: This document forms part of an EC evaluation data package and should not be read in isolation. Registration must not be granted on the basis of this document.

Figure 6.3

Proposed metabolic pathway of methomyl in the monkey

[ ] proposed

WARNING: This document forms part of an EC evaluation data package and should not be read in isolation. Registration must not be granted on the basis of this document.

**B.6.2 Acute toxicity, irritancy and sensitisation (IIA 5.2.1)****B.6.2.1 Acute oral toxicity (IIA 5.2.1)**

<b>Report</b>	Sarver, J.W. (1991a). Acute oral toxicity study with DPX-X1179-394 in male and female rats. Unpublished DuPont Report No. HLR 661-91 (in-life phase: 19 March 1991 to 17 April 1991).
<b>Test facility</b>	DuPont Haskell Laboratory, P.O. Box 50, Elkton Road, Newark, Delaware, 19714 USA.
<b>Guidelines</b>	Directive 92/69/EEC Method B.1 $\cong$ OECD Guideline 401.
<b>Deviations from guidelines</b>	$\pm$ 20% of the mean weight variation on the day of dosing was not determined. This minor deviation does not compromise the validity of the study.
<b>GLP</b>	Yes (laboratory inspected by the United States Environmental Protection Agency).
<b>Test substance</b>	DXP-X1179
<b>Batch</b>	X1179-394
<b>Purity</b>	98.35%
<b>Appearance</b>	White solid
<b>Test System:</b>	Rat: CrI:CD BR (10/sex/dose).
<b>Doses:</b>	20, 40 and 80 mg/kg bw.
<b>Vehicle:</b>	Deionised water.
<b>Acceptable</b>	Yes.

**Materials and Methods**

Groups of CrI:CD BR rats (10/sex/dose) were administered a single oral dose of methomyl by gavage at dose levels of 20, 40, and 80 mg/kg bw suspended in deionised water. The animals were observed for clinical signs of toxicity, body weight effects, and mortality for up to 14 days after dosing. At least three rats/sex/dose level that were either found dead or sacrificed by design were examined for gross pathological changes.

**Results**

<b>Dose (mg/kg bw)</b>	<b>Mortalities</b>	
	<b>Males</b>	<b>Females</b>
20	2/10 (day 1) <sup>a</sup>	1/10 (day 1)
40	5/10 (day 1)	8/10 (day 1)
80	10/10 (day 1)	10/10 (day 1)

Key: a) all deaths occurred within 1 day of dosing.

Clinical signs of toxicity most often observed in male and female rats dosed at 20 and 40 mg/kg bw included tremors, low posture, and salivation. These clinical signs of toxicity were only apparent one hour after dosing. All rats dosed at 80 mg/kg bw exhibited convulsions and were found dead after dosing. There were no test substance-related body weight effects noted. Gross pathological examination of the decedents revealed lung effects (expanded, bloody fluid), skin staining (white/red neck and face), oral cavity effects (white stain or discharge) and periocular effects (chromodacryorrhea). No gross abnormalities were detected in the surviving animals at the scheduled sacrifice.

## Conclusions

The acute oral LD50 value was 32 mg/kg bw for both males and females. Therefore, based on this study the test material is classifiable as 'Toxic' via the oral route according to EC criteria.

(Sarver, 1991a)

### B.6.2.2 Acute dermal toxicity (IIA 5.2.2)

<b>Report</b>	Sarver, J.W. (1991b). Acute dermal toxicity study of DPX-X1179-394 in rabbits. Unpublished DuPont Report No. HLR 455-91 (in-life phase: 29 May 1991 to 12 June 1991).
<b>Test facility</b>	DuPont Haskell Laboratory, P.O. Box 50, Elkton Road, Newark, Delaware, 19714 USA.
<b>Guidelines</b>	Directive 92/69/EEC Method B.3 $\equiv$ OECD 402.
<b>Deviations from guidelines</b>	$\pm$ 20% of the mean weight variation on the day of dosing was not determined. Necropsy was not conducted at the conclusion of the study. These minor deviations do not compromise the validity of the study.
<b>GLP</b>	Yes (laboratory inspected by the United States Environmental Protection Agency).
<b>Test substance</b>	DPX-X1179
<b>Batch</b>	X1179-394
<b>Purity</b>	98.35 %
<b>Appearance</b>	White solid
<b>Test System:</b>	Rabbit: New Zealand white (5/sex).
<b>Doses:</b>	2000 mg/kg bw (24 hour exposure).
<b>Vehicle:</b>	Moistened with deionised water to form a paste.
<b>Acceptable</b>	Yes.

## Materials and methods

A single dose of methomyl was applied to the shaved intact skin of New Zealand white rabbits at a dose of 2000 mg/kg bw. The application site was occluded for 24 hours after which the test substance was removed. The rabbits were observed for clinical signs, body weight effects, and mortality for 14 days following application.

## Results

No mortalities were observed. Slight erythema (grade 1 on the Draize scale) was observed in 2 rabbits on day 1 only. All rabbits appeared normal by day 2 and throughout the remainder of the study. Slight weight loss (up to 3% of initial body weight) occurred in some rabbits the day following treatment. Since there was no evidence of systemic toxicity in these rabbits, pathological examinations were not performed.

## Conclusions

The dermal LD50 value was greater than 2000 mg/kg bw for both male and female rabbits. Therefore, the test material is not classifiable via the dermal route according to EC criteria.

(Sarver, 1991b)

**B.6.2.3 Acute inhalation toxicity (IIA 5.2.3)**

<b>Report</b>	Panepinto, A.S. (1991). Acute inhalation toxicity study with DPX-X1179-427 in rats. Unpublished DuPont Report No. HLR 678-91 (in-life phase: 31 July 1991 to 26 august 1991).
<b>Test facility</b>	DuPont Haskell Laboratory, P.O. Box 50, Elkton Road, Newark, Delaware, 19714 USA.
<b>Guidelines</b>	Directive 92/69/EEC Method B.2 $\equiv$ OECD 403.
<b>Deviations from guidelines</b>	$\pm$ 20% of the mean weight variation on the day of dosing was not determined. Chamber humidity exceeded the range of 30-70%. These minor deviations do not compromise the validity of the study.
<b>GLP</b>	Yes (laboratory inspected by the United States Environmental Protection Agency).
<b>Test substance</b>	DPX-X1179
<b>Batch</b>	X1179-427
<b>Purity</b>	97.7%
<b>Appearance</b>	White solid
<b>Test System:</b>	Rat: CrI:CD Br (10/sex/dose).
<b>Doses:</b>	Dose levels are presented in the tabulated results.
<b>Test material<sup>a</sup></b>	Aerosol (4 hour nose-only exposure).
<b>Vehicle:</b>	Distilled water
<b>Acceptable</b>	Yes.

**Materials and methods**

Groups of CrI:CD BR rats (5/sex/dose) were exposed to methomyl at dry weight concentrations of 0.137, 0.181, 0.182, 0.232 or 0.326 mg/l for 4 hours (nose-only exposure). The test substance was suspended in distilled water to create an aerosol. The animals were observed for clinical signs of toxicity, body weight effects and mortality for up to 14 days after dosing. Rats that were found dead or survived the observation period were examined for gross pathological changes.

**Results**

The particle distribution and chamber conditions are presented below:

Gravimetric Conc (mg/l)	Analysed <sup>a</sup> Conc (mg/l)	MMAD <sup>b</sup> (GSD) <sup>c</sup> ( $\mu$ m)	Particle size distribution by mass (%)			Temp (°C)	Relative humidity (%)
			<1 $\mu$ m	<3 $\mu$ m	<10 $\mu$ m		
0.137	0.126	d	d	d	d	24-25	70-83
0.181	0.160	1.3 (2.3)	40	84	99	23-24	76-93
0.182	0.179	1.3 (2.9)	40	79	98	24-24	74-92
0.232	0.215	3.8 (2.4)	6.2	38	85	22-23	89-100
0.326	0.304	2.7 (2.5)	14	55	93	21-23	99-100

Key: a) Chromatographic analysis of the filter eluent. b) Mass Median Aerodynamic Diameter. c) Geometric Standard Deviation. d) Not determined. e) Airflow ranged from 14.4-22.4 l/min.



The number of deaths and the time to death are presented below:

Gravimetric/Analysed Concentrations (mg/l)	Mortalities	
	Males	Females
0.137/0.126	0/5	0/5
0.181/0.160	0/5	0/5
0.18/0.179	0/5	1/5 (day 1)
0.232/0.215	3/5 (day 1) <sup>a</sup>	3/5 (day 1)
0.326/0.304	3/5 (day 1)	4/5 (day 1)

Key: a) Time of death.

The clinical signs of toxicity included nasal discharge, salivation, diarrhoea, lethargy, ocular and/or nasal discharge(s), and wet and/or stained fur. Animals exposed to concentrations where death occurred also exhibited abnormal gait or mobility, tremors, hyperactivity, hyper-reactivity, muscle fasciculations, and hunched or low posture. Corneal opacity was observed in one animal exposed to 0.232 mg/l. By the first day post exposure, most of the animals exhibited weight loss. The animals started to gain weight by the second day post exposure and despite some transient weight losses, experienced an overall gain by the end of the recovery period. Gross abnormalities found in rats that were found dead included expanded lungs and oedema of the pleural cavity. No test substance-related gross lesions were observed at necropsy in rats surviving the 14-day recovery period.

## Conclusions

Based on the data (gravimetric/analysed), the acute inhalation LC50 value appears to be less than 0.232/0.215 mg/l males and female rats (3/5 males and 3/5 females dead at this dose level). Probit analysis of the analysed results gives a combined LC50 of 0.2483 mg/l and a LC50 of 0.234 mg/l for females. Therefore, the test substance is classifiable as 'Very Toxic' via the inhalation route according to EC criteria (concentrations  $\leq 0.25$  mg/l for an aerosol is classifiable as Very Toxic) based on the analysed data.

*The company has calculated the inhalation LC50 value for methomyl in rats to be 0.258 mg/l for males and female rats based on probit analysis of the gravimetric data. This would result in a classification of 'Toxic' according to EC criteria.*

(Panepinto, 1991)

**B.6.2.4 Skin irritancy (IIA 5.2.4)**

<b>Report</b>	Sarver, J.W. (1993). Primary dermal irritation study with DPX-X1179-394 in rabbits. Unpublished DuPont Report No. HLR 563-93 (in-life phase: 21 July 1993 to 24 July 1993).
<b>Test facility</b>	DuPont Haskell Laboratory, P.O. Box 50, Elkton Road, Newark, Delaware, 19714 USA.
<b>Guidelines</b>	Directive 92/69/EEC Method B.4. $\cong$ OECD 404 (1981).
<b>Deviations from guidelines</b>	None.
<b>GLP</b>	Yes (laboratory inspected by the United States Environmental Protection Agency).
<b>Test substance</b>	DPX-X1179
<b>Batch</b>	X1179-427
<b>Purity</b>	98.35%
<b>Appearance</b>	White solid
<b>Test System:</b>	Rabbit: six New Zealand White females.
<b>Doses:</b>	0.5 g (4 hour exposure/semi-occlusive dressing).
<b>Vehicle:</b>	Moistened with deionised water.
<b>Acceptable</b>	Yes.

**Materials and methods**

Skin reactions were scored using the Draize scale at approximately 1, 24, 48 and 72 hours after removal of the dressings.

**Results**

There were no skin reactions, body weight effects or clinical signs in any animals.

**Conclusions**

The test substance is not classifiable as a skin irritant according to EC criteria.

(Sarver, 1993)

**B.6.2.5 Eye irritancy (IIA 5.2.5)**

<b>Report</b>	Sarver, J.W. (1991c). Primary eye irritation study with DPX-X1179-425 in rabbits. Unpublished DuPont Report No. HLR 280-91 (in-life phase: 19 March 1991 to 1 April 1991).
<b>Test facility</b>	DuPont Haskell Laboratory, P.O. Box 50, Elkton Road, Newark, Delaware, 19714 USA.
<b>Guidelines</b>	Directive 92/69/EEC Method B.5 $\equiv$ OECD 405.
<b>Deviations from guidelines</b>	<sup>a</sup> 10 mg of test material was tested. This deviation does not compromise the validity of the study.
<b>GLP</b>	Yes (laboratory inspected by the United States Environmental Protection Agency).
<b>Test substance</b>	DPX-X1179 90SP (formulation/Lannate 90)
<b>Batch</b>	X1179-425
<b>Purity</b>	92.4%
<b>Appearance</b>	White solid
<b>Test System:</b>	Rabbit: six New Zealand White males.
<b>Doses:</b>	10 mg of test material was tested (a weight corresponding to 0.017 ml).
<b>Vehicle:</b>	None
<b>Acceptable</b>	Yes.

Key: a) The acute lethality of methomyl precluded the use of 0.1 ml (approximately 60 mg). A range-finding study determined that 10 mg would not be fatal. An ocular dose of approximately 15 mg (0.025 ml) was fatal (20 minutes after treatment) to a female rabbit with a body weight of 2.655 kg (approximately 5.6 mg/kg bw).

**Materials and methods**

Ocular reactions were scored on the Draize scale at 24, 48 and 72 hours and seven days after dosing.

**Results**

Time	Corneal opacity						Iridial reactions						Conjunctiva											
													Redness						Chemosis					
No	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
1 h	0	0	0	0	0	0	1	1	1	0	1	0	1	1	1	1	1	1	0	0	0	0	0	0
24 h	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1
48 h	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	1	0	0	0	0	0	0	0
72 h	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	0	0	0	0	0
7 d	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-
Mean 24-72 hours	0.0						0.0						0.72						0.33					

All signs of irritation had resolved by 7 days post treatment. Pupillary constriction of the treated eye, incoordination, tremors, convulsions, profuse salivation, lethargy, rales, and/or fasciculations were observed in the treated rabbits between approximately 1-20 minutes following treatment. By 1 hour following treatment, pupillary constriction was still evident in the treated rabbit eyes. These clinical signs of toxicity are typical of the inhibition of cholinesterase activity and are considered to be indicative of neurotoxicity. These effects were not present the day after treatment.

## Conclusions

The test substance is not classifiable as an eye irritant according to EC criteria. However, it is noteworthy that the test substance is extremely toxic via the ocular route.

(Sarver, 1991c)

### B.6.2.6 Skin sensitisation (IIA 5.2.6)

<b>Report</b>	Armondi, S. (1991). Closed-patch repeated insult dermal sensitisation study (Buehler method) with DPX-X1179-394 in Guinea pigs (Revision 1). Unpublished DuPont Report No. HLO 345-91 (in life phase: 26 March 1991 to 25 April 1991).
<b>Test facility</b>	Pharmakon Research International, Inc., P.O. Box 313, Waverly, Pennsylvania, 18471 USA.
<b>Guidelines</b>	Directive 92/69/EEC Method B.6 $\equiv$ OECD 406 (1981).
<b>Deviations from guidelines</b>	None.
<b>GLP</b>	Yes (laboratory inspected by the United States Environmental Protection Agency).
<b>Test substance</b>	DPX-X1179
<b>Batch</b>	X1179-394
<b>Purity</b>	98.35%
<b>Appearance</b>	White solid
<b>Test method</b>	Buehler test (an appropriate method because of the potential toxicity following injections).
<b>Test System</b>	Duncan Hartley Guinea Pig.
<b>Group size</b>	20 test (10/sex) & 10 negative control animals (5/sex)
<b>Positive control</b>	1-chloro-2,4-dinitrobenzene (3 males and 2 females).
<b>Induction phase</b>	1 <sup>st</sup> , 2 <sup>nd</sup> & 3 <sup>rd</sup> induction: 300 mg of the test material moistened with 0.3 ml of distilled water.
<b>Challenge phase</b>	1 <sup>st</sup> challenge: 300 ml of the test material moistened with 0.3 ml of distilled water.
<b>Acceptable</b>	Yes.

## Materials and methods

The concentrations used in the induction and challenge phases were based on the results of preliminary studies (1%, 10% 50% and 100% w/v of test substance in 80% ethanol).

In the main study, an induction application was applied to clipped intact skin of each test animal using a Hill Top Chamber covered by occluded protective device. This induction procedure was performed once a week for 3 consecutive weeks, for a total of three 6-hour treatments with the test material. Following the same procedures, 3 male and 2 female guinea pigs were treated with 0.3 ml suspension of the positive control DNCB (0.3% in 80% ethanol). An additional group of 10 guinea pigs (5 male and 5 female) was treated with 80% ethanol to serve as a negative control. After approximately a 6-hour exposure period, the protective devices were removed from each animal. Irritation responses were scored approximately 24 and 48 hours after each treatment.

Two weeks after the last induction treatment, the test animals were challenged with 0.3 ml of the neat test material moistened with 0.3 ml distilled water. The challenge applications were placed onto previously untreated clipped skin on the back of each

animal using the same procedure as used with the induction applications. The positive control guinea pigs were treated by applying 0.3 ml of a 0.3% suspension of DNCB in acetone. The negative control animals were treated with 0.3 ml of the vehicle (acetone) on the left flank and 300 mg of the neat test material, moistened with 0.3 ml distilled water, on the right flank. After approximately a 6-hour exposure period, the protective devices were removed from the animals.

Twenty-one hours after exposure, a commercial depilatory was placed on the test sites and surrounding areas for no more than 30 minutes. The skin of each animal was washed with warm water to remove the depilatory then gently patted dry. Irritation responses were scored 3-hours after depilation (24-hours post exposure). The grading was repeated 24-hours later (48-hour post exposure).

## Results

During the induction phase, no substance-induced skin reactions were observed in the test or negative control animals. No skin reactions were observed in the test or negative control animals following the challenge applications. Appropriate results were obtained with the positive control animals.

## Conclusions

The test substance is not classifiable as a skin sensitizer according to EC criteria.

(Armondi, 1991)

### B.6.2.7 Summary of acute toxicity, irritancy and skin sensitisation

The results of the acute toxicity studies are presented in Table 6.12. All of these acute studies were performed in the early 1990s (methomyl purity: 92.4-98.35%).

Methomyl has a high order of acute toxicity in experimental animals via the oral, ocular and inhalation routes of exposure, but it has a relatively low order of acute toxicity via the dermal route. The human volunteer study (B.6.8.3) reported a human fatality at an estimated acute oral dose of approximately 12 mg/kg bw.

Methomyl is not an eye or skin irritant and does not cause skin sensitisation based on a Buehler assay. The company did not justify the test method but it is possible the most appropriate method based on methomyl toxicity.

It is noteworthy that the methomyl (purity: 92.4%) tested in the eye irritation test was extremely toxic via the ocular route. An ocular dose of approximately 15 mg (0.025 ml) was fatal (20 minutes after treatment) to a female rabbit with a body weight of 2.655 kg (approximately 5.6 mg/kg bw). While an ocular dose of 10 mg (0.017 ml) or approximately 3.8 mg/kg bw induced cholinergic effects in rabbits approximately 1-20 minutes after treatment.

Although the results of the acute oral and inhalation studies are borderline, the weight of evidence indicates that methomyl should be classifiable as Very Toxic (by inhalation and Toxic (if swallowed) based on the submitted GLP compliant studies. Additional oral toxicity studies (see footnote to table 6.12) indicate a higher

classification might be appropriate and the ECB classification meeting will wish to consider the overall data base.

Table 6.12. Summary of the acute toxicity and irritancy of methomyl

Study	Species	Results/comments	Classification	Reference
Acute oral	Rat	LD50: 32 mg/kg bw in both sexes.	Classified: Toxic (R 25)	Sarver, 1991a
Acute oral	Rat	LD50: 17-23 mg/kg bw (sex not specified)	Classified: Very Toxic (R 28)	<sup>a</sup> Hawkins <i>et al</i> , 1991
Acute dermal	Rabbit	LD50: >2000 mg/kg bw in both sexes	Unclassified	Sarver, 1991b
Acute inhalation	Rat	LC50: 0.232/0.215 mg/l (gravimetric/analysed) in both sexes (4 hour exposure).	Classified: Very Toxic (R 26)	Panepinto, 1991
Skin irritation	Rabbit	Non-irritant.	Unclassified	Sarver, 1993
Eye irritation	Rabbit	Slight irritant.	Unclassified	Sarver, 1991c
Skin sensitisation	Guinea pig	Negative in a Buehler test.	Unclassified	Armondi, 1991

Key: a) The metabolism study cited these acute LD50 values (Haskell Lab Report 499-79), acute oral LD50 values 17-45 mg/kg bw are cited in the human volunteer study (B.6.8.3) and an acute oral LD50 value of approximately 20 mg/kg bw is cited in a 2001 JMPR Report (Pesticide residues in food). The notifier has pointed out that these data were obtained from a non-guideline and non-GLP study conducted in 1966 using fasted animals and peanut oil as the vehicle. The notifier maintains that the 1991 GLP study using an aqueous vehicle is more representative of potential human exposure.

(Anon, 2004)

### B.6.3 Short-term toxicity (IIA 5.3)

Three short-term feeding studies have been conducted in rats and two in mice. These studies include one long-term study in the rat and one in the mouse that were prematurely terminated at weeks 35 and 23, respectively (sections B.6.3.1c and B.6.3.2b). A 90-day study and 2-year study have been conducted in dogs.

#### B.6.3.1 Oral studies in rats (IIA 5.3.1, 5.3.2)

a)

<b>Report</b>	Busey, W.M. (1966). Three-month dietary administration - rats insecticide 1179. Unpublished DuPont Report No.: Hazleton 1466. Published: No. (in-life phase: 8 July 1965 to 7 October 1965).
<b>Test facility</b>	Hazleton Laboratories America, Inc., 9200 Leesburg Turnpike, Vienna, Virginia, 22580 USA.
<b>Guidelines</b>	Directive 87/302/EEC Part B: Subchronic toxicity - rodent
<b>Deviations from guidelines</b>	± 20% of the mean weight variation on test day 0 was not determined. Length of acclimation period was not described in report. An ophthalmology exam was not conducted. The following haematology/clinical chemistry parameters were not evaluated: clotting potential, electrolyte balance, kidney function, liver function, and carbohydrate metabolism. Five males and 5 females per test group were sacrificed after 2 months of test substance administration. The following tissues were not collected/evaluated: aorta, oesophagus, rectum and lymph node.
<b>GLP</b>	No
<b>Test material</b>	DPX-X1179

<b>Batch No</b>	X1179
<b>Purity</b>	100% (as stated by report)
<b>Appearance</b>	White crystalline powder
<b>Acceptable</b>	Not acceptable for regulatory purposes on a stand-alone basis (but contains relevant scientific information).

## Materials and methods

Four groups of Crl:CD BR rats (10 rats/sex/group) were administered methomyl (purity 100%) in diet at concentrations of 0, 10, 50 or 250 ppm for 13 weeks. A fifth group of male and female rats was fed 125 ppm for 6 weeks and 500 ppm for the remainder of the study. In this group only, 5 animals/sex were sacrificed at 2 months and the remainder were sacrificed at study termination (an identical number of stock rats were sacrificed to act as controls).

The animals were examined daily for clinical signs and mortality. General appearance, body weight, body weight gain and food consumption were recorded weekly. Haematology examinations (tail vein samples) and urinalysis were conducted prior to the start of the study and at 1, 2 and 3 months (5 rats/sex/dose group). Plasma and red blood cell cholinesterase determinations were carried out on the animals sacrificed at 2 months (5 rats/sex). At termination, plasma and red blood cell cholinesterase determinations were carried out on the animals in the control and 250 ppm groups (5 rats/sex) and on the remaining animals (5 rats/sex) in the 125/500 ppm group (no further details provided). At necropsy, all rats received a gross examination and selected organs were removed and weighed (sacrificed by exsanguination). A comprehensive list of tissues and organs were removed and fixed for histopathological examination. Preserved tissues from the control and the 250 ppm group and bone marrow sections from the 10 and 50 ppm groups were examined microscopically (10 rats/sex/dose group).

## Results

Analysis of the diet for concentration, homogeneity and stability was not reported.

No deaths were observed during the study. The only clinical sign of toxicity noted was transient tremors observed in one 250 ppm female at week 13. Growth was suppressed in the 250 ppm males and in the 500 ppm males and females) during weeks 7-13 (significantly different from control values in the 500 ppm males only. Food consumption for the 500 ppm males was lower but not significantly different from the controls. Food consumption for the 250 ppm males and 500 ppm males and females was slightly lower compared with controls.

Minor haematological changes were noted during the study. The mean haemoglobin value was significantly reduced in the 250 ppm males at 2 months and the mean red blood cell count was significantly reduced in the 250 ppm females at 3 months. At the 2 month sampling time point, the mean plasma cholinesterase levels in control and 125 females appeared to be 3-fold higher than would be expected (the report concluded this was a technical error). No significant differences were found in cholinesterase activity at 3 months or in the urinalysis values at any time point.

No test substance-related gross findings were observed at necropsy. Significantly decreased liver weights and increased brain/body weight ratios were observed for the 125/500 ppm and 250 ppm males sacrificed after 13-weeks. These differences from control values were related to lower body weights of these animals when compared to controls. Microscopic examination of tissues from the 250 ppm rats revealed moderate erythroid hyperplasia in the bone marrow of all male rats in this group.

Table 6.13 Mean organ weights in male rats at 13 weeks

Dose (ppm)	0	10	50	<sup>a</sup> 125/500	250
Absolute liver weight (g)	18.83	18.51	17.90	15.67*	16.38*
Relative liver weight	3.72	3.83	3.81	3.60	3.68
Absolute brain weight (g)	2.05	2.10	2.10	2.12	2.13
Relative brain weight	0.404	0.438	0.452	0.492*	0.481*

Key: a) 125 ppm for 6 weeks and 500 ppm for 7 weeks.

## Conclusions

Under the conditions of this study, a NOAEL of 50 ppm (approximately 3.6 mg/kg bw/day) was determined for male rats based on decreased body weight/food consumption at concentrations  $\geq$  250 ppm and an increased incidence of erythroid hyperplasia in the bone marrow of males at 250 ppm. A NOAEL of 50 ppm (approximately 4.1 mg/kg bw/day) was determined for female rats based on the lower RBC and haemoglobin at 250 ppm and body weight/food consumption effects at 500 ppm. No details were provided on the analytical methods or methodology used for the cholinesterase determinations. Brain cholinesterase activity was not evaluated in this study.

(Busey, 1966)

b)

<b>Report</b>	Cox, R.H. (1979a). A subacute (90 day) evaluation of Nudrin® in the Fischer-344 rat for Shell Oil Company. Unpublished DuPont Report No: 52B 101-630-78 (in-life phase: 15 November 1978 to 16 February 1979).
<b>Test facility</b>	Toxicology and Pathology Services, Inc., P.O. Box 333, Mt. Vernon, Indiana 47820 USA.
<b>Guidelines</b>	Directive 87/302/EEC Part B: Subchronic toxicity - rodent.
<b>Deviations from guidelines</b>	Deviations: $\pm$ 20% of the mean weight variation on test day 0 was not determined. An ophthalmology exam was not conducted. The following tissues were not collected/evaluated: aorta and peripheral nerve.
<b>GLP</b>	No
<b>Test material</b>	DPX-X1179
<b>Batch No</b>	SD 14999-Tech
<b>Purity</b>	Not specified
<b>Appearance</b>	White crystalline powder
<b>Acceptable</b>	Not acceptable for regulatory purposes on a stand-alone basis (but contains relevant scientific information). There are some reservations on the sampling method and timing of the blood samples for the cholinesterase determinations and the high mortality rate.

## Materials and methods



Groups of Fischer rats (20 rats/sex/group) were administered diet containing methomyl (unspecified purity) dissolved in an acetone/corn oil mixture (2% w/w) for 13 weeks. The dosing regimen is presented in Table 6.14.

WARNING: This document forms part of an EC evaluation data package and should not be read in isolation. Registration must not be granted on the basis of this document.

Table 6.14. Dosing regimen

Group	No of rats/sex	Treatment (13 weeks unless stated otherwise)	
		Males (ppm)	Females (ppm)
I	20	0	0
II	20	20 (weeks 1-9) 1000 (weeks 10-13)	27 (weeks 1-9) 1500 (weeks 10-13)
III	20	50 (weeks 1-4) 800 (weeks 5-13)	68 (weeks 1-4) 1080 (weeks 5-13)
IV	20	100	135
V	20	200	270
VI	20	400	540
VII	20	600	810

The animals were observed twice daily for clinical signs and mortality. Body weights and food consumption were recorded weekly. Haematological evaluations (10/sex/dose) were carried out prior to the start of the study (tail vein), on day 42 (orbital sinus) and at termination (tail vein). Serum chemistry evaluations (10/sex/dose) were carried out prior to the start of the study (inferior vena cava), on day 42 (orbital sinus) and at termination (inferior vena cava). Plasma and red blood cell cholinesterase activity was determined in blood samples taken shortly before dosing at weeks 1, 3, 6 and 13. All blood samples were taken from the orbital sinus and the activities were determined using a modified Ellman method. Urinalysis (10/sex/dose) was carried out on 18-hour samples on day 45 and at termination. The animals were hydrated (40 ml of water/kg) to induce urination prior to the 45 day collection but not at termination. At necropsy, all rats received a gross examination and selected organs were removed and weighed (sacrificed using chloroform). A comprehensive list of organs and tissues and unusual lesions were removed for histopathological examination.

## Results

Analysis of the diet confirmed that the concentrations of the test article were acceptable. The test material was reported to be stable for up to 3 weeks in diet at temperature below 25 °C and for 2.5 months in a freezer. Fresh diet was prepared weekly and they were mixed to obtain homogeneity.

The mortality data are presented in Table 6.15.

Table 6.15. Mortality data

Group	Males		Females	
	Dose ppm (13 weeks duration)	FD/SM (time of death)	Dose ppm (duration)	FD/SM (time of death)
I	0	0/20	0	0/20
II	20 (weeks 1-9) 1000 (weeks 10-13)	0/20	27 (weeks 1-9) 1500 (weeks 10-13)	0/20 (weeks 1-6) 3/20 (during week 7)
III	50 (weeks 1-4) 800 (weeks 5-13)	0/20 2/20 (week 7)	68 (weeks 1-4) 1080 (weeks 5-13)	0/20 (weeks 1-6) 4/20 (during week 7)
IV	100	0/20	135	3/20 (1 during week 2 & 2 during week 7)
V	200	0/20	270	7/20 (1 during week 2 & 6 during week 7)
VI	400	0/20	540	5/20 (1 during week 2 & 4 during week 7)
VII	600	0/20	810	7/20 (during week 7)

Key: a) Found dead/sacrificed moribund.

A total of 31 animals were found dead or sacrificed moribund during the study. Two male rats (Group III) were found dead following hydration during week 7 (it is not clear in the report how the water was administered, i.e. imbibed or gavage). One of these males exhibited clonus convulsions and loss of righting reflex prior to sacrifice. Three females died during week 2 and twenty-six females died during week 7 shortly after hydration. Gross and microscopic findings in the three females that died during week 2 and one female (not dosed with water) that died during week 7 (Group VI) indicated that the major causes of death in these animals was gastritis, enteritis and/or colitis or gastrointestinal distress. Twenty females that died during week 7 had watery fluid in their pleural cavities and darkened lungs. In addition, the report stated these 20 females had oesophageal ruptures due to attempts at regurgitation after water loading. However, it is possible that these animals died due to error during gavage administration. The cause of death in five females (one in Group II, III, IV, VI & VII) appeared to be related to systemic infections and was also attributed to water loading by the report.

There were some significant decreases in mean body weight in Group II males (weeks 10 & 11) associated with a decrease in food consumption. The mean body weights of females are presented in Table 6.16.

Table 6.16 Mean body weights of females (g)

Group (ppm)	I (0)	IV (135)	V (270)	VI (540)	VII (810)	III (68/1080) <sup>a</sup>	II (27/1500) <sup>b</sup>
1	118.0	116.4	114.2	111.2	114.0	118.1	114.7
2	128.4	131.1	129.3	127.1	127.2	129.7	128.2
3	138.4	138.4	137.8	130.5*	134.5	139.2	137.2
4	149.1	146.5	147.9	141.0*	145.1	147.0	144.7
5	156.9	155.4	156.6	147.9*	153.0	148.7*	154.4
6	155.9	157.1	159.6	146.5*	156.4	154.1	155.1
7	159.1	159.1	157.4	156.1	160.1	157.2	158.0
8	163.0	157.9	157.3	157.5	161.5	158.9	159.2
9	169.7	162.8	163.2	165.9	164.9	164.3	166.6
10	176.9	166.8*	169.5*	170.4	166.3*	170.9	159.9*
11	179.5	168.9*	170.2*	173.1	170.4*	170.8*	165.5*
12	182.6	174.7*	176.3	177.5	174.8*	178.9	174.5*
13	180.1	175.2	174.5	177.2	173.8	180.9	175.8

Key: a) 68 ppm weeks 1-4 & 1080 ppm weeks 5-13. b) 27 ppm weeks 1-9 & 1500 ppm weeks 10-13.

Although there were no clear dose response relationships, decreases in mean body weight were observed in females at all dose levels. Food consumption was significantly increased in females at several time points in all groups except Group II.

The changes in the red blood cell parameters are presented in Table 6.17 and 6.18.

Table 6.17 Mean haematological parameters in males

Group (ppm)	I (0)	IV (100)	V (200)	VI (400)	VII (600)	III (50/800) <sup>a</sup>	II (20/1000) <sup>b</sup>
Males (week 6)							
MCV (cubic microns)	56.5	57.4	58.5*	57.7	58.3*	57.4	57.1
Haemoglobin (g/dl)	17.1	16.7	12.7*	15.3*	14.8*	15.9*	16.4
Males (week 13)							
Red blood cells (millions)	5.17	4.88	5.39	4.97	4.09*	4.75	5.02
MCV (cubic microns)	53.8	53.9	54.7	53.8	55.9*	53.2	54.3
Haemoglobin (g/dl)	16.5	15.5	16.0	14.1*	19.0*	14.4*	15.7
Reticulocytes (per 5000 rbc)	13.8	20.3	16.6	17.5	21.5*	25.5*	25.8*
Platelets (thousands)	1701.8	1979.0*	2010.5*	2029.5*	2137.5*	1771.0	1869.0

Key: a) 50 ppm weeks 1-4 & 800 ppm weeks 5-13. B) 20 ppm weeks 1-9 & 1000 ppm weeks 10-13.

The mean haemoglobin levels were significantly reduced at 200-50/800 ppm in males at 6 weeks and 400-50/800 ppm at 13 weeks.

Table 6.18 Mean haematological parameters in female

Group (ppm)	I (0)	IV (135)	V (270)	VI (540)	VII (810)	III (68/1080) <sup>a</sup>	II (27/1500) <sup>b</sup>
Females (week 6)							
Red blood cells (millions)	5.70	5.57	5.52	5.26	5.08*	5.38	5.34
MCV (cubic microns)	57.4	58.9	59.6	58.5	60.4*	58.2	58.2
Females (week 13)							
Red blood cells (millions)	5.68	5.56	5.43	5.27	4.62*	5.23	4.95
HCT (%)	26.8	27.1	26.0	24.8	21.1*	24.9	23.9
Haemoglobin (g/dl)	17.1	17.4	16.8	15.9*	15.7*	15.8*	15.2*
Reticulocytes (per 5000 rbc)	16.2	17.8	24.4*	35.1*	32.1*	38.9*	49.5*
Platelets (thousands)	1397.0	1476.0	1471.0	1413.0	1312.0	1280.5	1197.5*

Key: a) 68 ppm weeks 1-4 & 1080 ppm weeks 5-13. b) 27 ppm weeks 1-9 & 1500 ppm weeks 10-13.

The mean haemoglobin levels were significantly reduced in females at 540-27/1500 ppm at 13 weeks only. The mean reticulocyte counts were significantly increased in 600, 50/800, and 20/1000 ppm males and in females at 200 ppm and above. The reticulocyte counts for the six-week time point were not reported.

Significantly reduced glucose levels were noted for male rats given 400, 600, and 50/800 ppm for 6 weeks, but not after 13 weeks. Glucose was also reduced in female rats at 540 ppm and above at 13 weeks. Urea was increased in 27/1500 ppm females at week 13. The report stated that no distinct or apparent test substance-related differences were observed in the results of the urinalysis conducted during week 7 or at termination (no summary or individual data were included in the report).

Significant reductions in plasma cholinesterase activity were noted in females at 810 ppm or greater, but similar changes were not observed in the male rats (Table 6.19). In contrast, there was an apparent treatment related decrease in RBC cholinesterase activity from the 50/800 ppm males at termination, but similar changes were not observed in the female rats (Table 6.20).

Table 6.19 Mean cholinesterase activity in males

Group (ppm)	I (0)	IV (100)	V (200)	VI (400)	VII (600)	III (50/800) <sup>a</sup>	II (20/1000) <sup>b</sup>
Male plasma cholinesterase activity (mcmoles substrate hydrolysed/ml/min)							
Week 1	0.52	0.42	0.44	0.38*	0.43	0.43	0.54
Week 3	0.40	0.38	0.40	0.41	0.37	0.29	0.43
Week 6	0.41	0.42	0.51	0.36	0.45	0.43	0.39
Week 13	0.46	0.57	0.57	0.62	0.59	0.49	0.38
Male erythrocyte cholinesterase activity (mcmoles substrate hydrolysed/ml packed cells/min)							
Week 1	1.50	1.23	1.73	1.40	1.50	1.43	1.39
Week 3	0.94	1.26	1.16	0.46*	0.86	0.90	1.35*
Week 6	0.80	0.81	1.28*	0.87	1.00	0.72	1.07
Week 13	1.07	1.22	1.09	1.08	1.13	0.66*	1.15

Key: a) 50 ppm weeks 1-4 & 800 ppm weeks 5-13. b) 20 ppm weeks 1-9 & 1000 ppm weeks 10-13.

Table 6.20 Mean cholinesterase activity in females

Group (ppm)	I (0)	IV (135)	V (270)	VI (540)	VII (810)	III (68/1080) <sup>a</sup>	II (27/1500) <sup>b</sup>
Female plasma cholinesterase activity (mcmoles substrate hydrolysed/ml/min)							
Week 1	1.09	1.07	1.05	1.01	0.76*	1.21	0.95
Week 3	1.48	1.74*	1.60	1.72*	1.44	1.67	1.58
Week 6	2.23	1.86*	2.15	2.20	2.31	2.49	2.17
Week 13	2.70	2.46	2.59	2.48	2.35*	2.15*	2.00*
Female erythrocyte cholinesterase activity (mcmoles substrate hydrolysed/ml packed cells/min)							
Week 1	1.12	1.96*	2.01*	2.08*	1.30	1.45	1.41
Week 3	1.07	1.20	1.63*	2.06*	1.20	1.12	1.30
Week 6	0.78	0.80	0.81	0.98	1.29*	0.70	0.91
Week 13	1.27	1.03	1.10	0.90*	1.09	1.44	1.40

Key: a) 68 ppm weeks 1-4 & 1080 ppm weeks 5-13. b) 27 ppm weeks 1-9 & 1500 ppm weeks 10-13.

The mean organ weight changes are presented in Table 6.21.

Table 6.21 Mean absolute organ weight (relative to body weight)

Males (g)							
Group (ppm)	I (0)	IV (100)	V (200)	VI (400)	VII (600)	III (50/800) <sup>a</sup>	II (20/1000) <sup>b</sup>
Spleen	0.49 (0.18)	0.52 (0.19)	0.52 (0.18)	0.53 (0.19)	0.55* (0.20*)	0.56* (0.21*)	0.58* (0.22*)
Females (g)							
Group (ppm)	I (0)	IV (135)	V (270)	VI (540)	VII (810)	III (68/1080) <sup>c</sup>	II (27/1500) <sup>d</sup>
Spleen	0.35 (0.22)	0.36 (0.22)	0.37 (0.23)	0.39 (0.24*)	0.41* (0.26*)	0.44* (0.27*)	0.50* (0.31*)
Kidney	1.24 (0.76)	1.23 (0.77)	1.26 (0.78)	1.27 (0.78)	1.29 (0.80*)	1.29 (0.79*)	1.29 (0.79*)
Liver	4.52 (2.76)	4.81* (2.78)	4.44 (2.84)	4.56 (2.91*)	4.73 (2.99*)	4.78 (2.94*)	4.73 (2.91*)

Key: a) 50 ppm weeks 1-4 & 800 ppm weeks 5-13. B) 20 ppm weeks 1-9 & 1000 ppm weeks 10-13.  
c) 68 ppm weeks 1-4 & 1080 ppm weeks 5-13. d) 27 ppm weeks 1-9 & 1500 ppm weeks 10-13.

Mean spleen weight was significantly increased in males at 600 ppm and above and in females at 540 ppm and above. Relative kidney weight was increased in females at 810 ppm and above and relative liver weight was increased at 540 ppm and above. No distinct or consistent test substance-related microscopic changes were noted in tissue sections evaluated.

## Conclusion

Under the conditions of this study, the NOAEL was determined to be 200 ppm (13.6 mg/kg bw/day) based on haematology changes at 400 ppm and greater. The NOAEL in females was 135 ppm (10.0 mg/kg bw/day) based on haematology changes at 270 ppm and greater. Brain cholinesterase activity was not evaluated in this study. In addition, the high mortality brought about by the administration of water (the blood sampling method may also be contributory factor) suggests that the conduct and results of this study are equivocal.

(Cox, 1979a)

c)

<b>Report</b>	Cox, R.H. (1980). Oncogenicity/chronic toxicity evaluation of Nudrin in Fischer-344 rats. Unpublished DuPont Report No.: TPS 52F-101-650-79 and accompanying Supplement Nos. 1 and No. 2. (in-life phase: April 1979 to December 1979).
<b>Test facility</b>	Toxicology and Pathology Services, Inc., P.O. Box 333, Mt. Vernon, Indiana 47820 USA.
<b>Guidelines</b>	Not applicable
<b>Deviations from guidelines</b>	The study was terminated at 35 weeks (no longer required for registration purposes).
<b>GLP</b>	No
<b>Test substance</b>	DPX-X1179 Technical.
<b>Batch</b>	SD 14999-Tech
<b>Purity</b>	Not specified
<b>Appearance</b>	White solid
<b>Acceptable</b>	Not acceptable for regulatory purposes on a stand-alone basis (but contains relevant scientific information).

### Materials and methods

Groups of Fischer-344 rats (80 or 160/sex/group) were administered diet containing methomyl at concentrations of 0 (160/sex), 35, 100, 300, 600 or 1200 ppm for 35 weeks. The first 20 controls/sex and the first 10/sex/test group were sacrificed at 6 months, the remaining animals were sacrificed at 35 weeks. Because of the premature termination of this study and the protocol amendments, the report did not contain the details or the results that would be expected in a standard carcinogenicity study.

Clinical observations were carried out at least twice daily. Each animal received a general examination weekly (for palpable masses and abdominal distension). Body weights and food consumption were recorded weekly for the first 13 weeks and then every two weeks thereafter. No ophthalmological examinations were carried out in this study. No clinical chemistry or urinalysis results were included in the report. Haematological changes in females at 35 weeks were reported. Plasma and erythrocyte cholinesterase activities in males and females at 35 weeks together with brain cholinesterase activities in male and females at 26 weeks were also reported. The pathology report contained macroscopic and microscopic evaluations of control animals (15/sex) and the gross findings in those animals that were found dead or had palpable masses at termination.

At 35 weeks, two blood samples (1 tail sample and 1 by cardiac puncture) were obtained for evaluation of the red blood cell parameters from 30 control females and 15 females per test group (these animals were not fasted). Blood samples (20 control rats/sex and 10/sex/test group) were obtained from the orbital sinus for the plasma and erythrocyte cholinesterase determinations (blood samples were stored at -20 °C until analysed by the Ellman method). The animals sacrificed at the six-months were fasted overnight and then killed by exposure to chloroform. The left hemisphere of the brain was quickly frozen for brain cholinesterase determinations (Ellman method).

## Results

There were no compound-related mortalities or clinical signs observed during this study. The changes in mean body weight, food consumption and food efficiency are presented in Table 6.22.

Table 6.22. Mean body weights, food intakes and food efficiency values at 35 weeks

Dose (ppm)	0	35	100	300	600	1200
Males						
Body weight (g)	374.7	385.9*	375.7	370.5	342.6*	312.3*
Food consumption (g/week)	126.4	121.8	122.8	124.1	121.0	113.7*
Food efficiency (g/kg bw/day)	48.3	45.1*	46.9	48.0	49.4*	51.6*
Females						
Body weight (g)	218.0	219.9	218.5	214.6	205.7*	192.4*
Food consumption (g/week)	90.2	90.3	93.7	90.5	90.0	88.7
Food efficiency (g/kg bw/day)	59.2	58.8	61.4	60.2	62.5*	65.9*

At dose levels of 600 and 1200 ppm for 35 weeks, the mean values for male body weights were 7% and 16% less, respectively, than controls. In females at those dose levels, the body weights were 6% and 12% less, respectively, compared to controls. The mean food consumption values (grams consumed per week) were significantly decreased at several weeks primarily at 300 ppm and above. However, mean food efficiency (grams/kg bw/day) was significantly increased at 600 ppm and above since body weights were significantly decreased.

Blood samples were obtained from the tip of the tail and by cardiac puncture from females (males were not evaluated). Statistical evaluation of the data revealed a significantly smaller group mean value for RBC counts for 1200 ppm females regardless of the sampling method. There was a concomitant significant increase in circulating reticulocytes for the 1200 ppm females for both samples. Additionally, there were significant increases in reticulocytes noted for the 300 and 600 ppm females for samples obtained from the tip of the tail.

Table 6.23. Mean haematological changes in females at 35 weeks

Dose (ppm)	0 (n = 30)	35 (n = 15)	100 (n = 15)	300 (n = 15)	600 (n = 15)	1200 (n = 15)
Tail samples						
<sup>a</sup> Erythrocyte count	8.19	8.12	7.96	7.81	8.05	7.48*
<sup>a</sup> Reticulocyte count	3.0	2.8	3.2	3.9*	3.8*	4.7*
Heart samples						
<sup>a</sup> Erythrocyte count	8.27	8.03	7.97	7.91	8.07	7.59*
<sup>a</sup> Reticulocyte count	3.2	3.1	3.2	3.4	3.5	4.6*

Key: a) Units not stipulated.

The mean cholinesterase values obtained at 26 weeks and 35 weeks are presented in Table 6.24. Significant decreases in RBC cholinesterase activity (>20%) were observed at 100 ppm and above in both sexes. The report stated that the effects on brain cholinesterase levels were equivocal (no reasons were given in the report). No conclusions were drawn from the plasma cholinesterase evaluation due to technical malfunction in the analysis. It is noticeable that there are marked differences between the sexes in the mean brain and RBC cholinesterase activities. There are also



statements in the study protocol(s) indicating that the animals were fasted for 24 hours before sacrifice at (i.e. the reliability of the brain cholinesterase determinations appears to be questionable).

Table 6.24. Mean plasma and erythrocyte cholinesterase values at 35 weeks and brain cholinesterase values at 26 weeks

Dose (ppm)	0 (n = 20)	35 (n = 10)	100 (n = 10)	300 (n = 10)	600 (n = 10)	1200 (n = 10)
<b>Males</b>						
<sup>a</sup> Plasma cholinesterase	3.5 (0.38) <sup>b</sup>	3.4 (0.22)	3.6 (0.19)	1.8* (0.15)	1.6* (0.34)	1.4* (0.18)
Erythrocyte cholinesterase	29.0 (3.43)	28.8 (3.93)	23.6* (5.79) ↓ 18.6%	22.5 (4.52) ↓ 22%	27.1 (3.29) ↓ 6.5%	12.0* (6.04) ↓ 60%
Brain cholinesterase (week 26)	18.0 <sup>c</sup> (3.12)	21.0 (3.41)	18.4 (8.06)	17.6 <sup>d</sup> (3.44)	19.12 (3.16)	14.6 (3.91) ↓ 18.9%
<b>Females</b>						
Plasma cholinesterase	1.9 (0.21) <sup>a</sup>	1.8 (0.26)	5.6* (0.21)	5.8* (0.34)	5.9* (0.18)	6.2* (0.26)
Erythrocyte cholinesterase	64.7 (6.41)	56.4* (4.0)	57.5* (5.04)	49.6* (6.36) ↓ 23%	50.5* (3.65) ↓ 21.9%	49.5* (4.85) ↓ 23%
Brain cholinesterase (week 26)	7.9 (4.36)	5.2 (4.19) ↓ 34%	4.4 (2.71) ↓ 44%	4.8 <sup>d</sup> (2.36) ↓ 39%	3.9* (3.27) ↓ 50.6%	6.6 (4.29) ↓ 16.5%

Key: a) Units of cholinesterase activity was defined as the amount of enzyme which will hydrolyse 0.1 micromole of thioester per minute at 37°C. b) Standard deviation. c) n = 18. d) n = 9.

There were no compound-related gross or histopathologic findings or organ weight changes noted at termination.

## Conclusions

Because of the equivocal results and absence of methodology details, NOAELs for plasma, erythrocyte and brain cholinesterase inhibition have not been determined for this study. Under the conditions of this study, a NOAEL of 300 ppm (estimated to be approximately 15.0 mg/kg bw/day) can be determined for body weight in both sexes based on decreased body weight at 600 ppm and above. A NOAEL of 100 ppm (estimated to be approximately 5.0 mg/kg bw/day) can be determined for red blood cell effects in females based on haematology changes at 300 ppm and above.

*The company claimed a NOAEL of 300 ppm (approximately 14.4 mg/kg bw/day) for males based on decreased body weight at 600 ppm and above and 100 ppm (approximately 6.4 mg/kg bw/day) for females based on haematology changes at 300 ppm and above.*

(Cox, 1980)

**B.6.3.2 Oral studies in mice (IIA 5.3.1, 5.3.2)**

a)

<b>Report</b>	C Cox, R.H. (1979b). A subacute (90 day) evaluation of Nudrin in the B6C3F1 mouse for Shell Oil Company. Unpublished DuPont Report No.: 52E 004-630-79 (in-life phase: 1979 ).
<b>Test facility</b>	Toxicology and Pathology Services, Inc., P.O. Box 333, Mt. Vernon, Indiana 47820 USA.
<b>Guidelines</b>	Directive 87/302/EEC Part B: Subchronic toxicity - rodent.
<b>Deviations from guidelines</b>	± 20% of the mean weight variation on test day 0 was not determined. An ophthalmology exam was not conducted. The following haematology/clinical chemistry parameters were not evaluated: a measure of clotting potential. The following tissues were not collected/evaluated: pituitary, thymus, aorta, gonads, uterus, rectum, bladder, peripheral nerve, sternum and bone marrow.
<b>GLP</b>	No
<b>Test material</b>	DPX-X1179
<b>Batch No</b>	SD 14999-Tech
<b>Purity</b>	Not specified
<b>Appearance</b>	White crystalline powder
<b>Acceptable</b>	Not acceptable for regulatory purposes on a stand-alone basis (but contains relevant scientific information).

**Materials and methods**

Groups of Crl:CD BR mice (20/sex/dose) were administered methomyl (unspecified purity) at dietary concentrations of 0, 75, 150, 300, 480, 600 (11 males and 9 females) or 960 ppm for 3 months.

All animals were observed twice daily for mortality and clinical signs of toxicity. Body weight and feed consumption were recorded weekly. Haematological and clinical chemistry parameters were evaluated at termination (tail samples). Urinalysis was carried out at termination. No cholinesterase determinations were carried out. All animals were subjected to gross examination at necropsy (sacrificed using chloroform). Selected organs were removed and weighed (brain, liver, heart, kidneys, adrenals, ovaries/with fallopian tubes, uterus and testes). Microscopic examinations of selected tissues and organs (brain heart, lungs, liver, spleen, kidneys and gonads) were carried out at all dose levels and an extended list of organs and tissues were examined at 0 and 960 ppm.

**Results**

The diets were prepared fresh twice a week and mixed for 1 hour to obtain homogeneity. Samples of the diet were retained for analysis but no results were included in the report.

No compound-induced deaths occurred during the study. There were no consistent compound-related clinical signs or effects on body weights or food consumption. The main haematological changes are presented in Table 6.25.

Table 6.25. Mean haematological changes at termination

Group (ppm)	0 (n=20)	75 (n=10)	150 (n=10)	300 (n=9)	480 (n=9/10)	600 (n=11/9)	960 (n=10)
Males (13 weeks)							
Red blood cells (millions)	4.28	4.31	4.23	4.17	4.31	5.36*	5.22*
Haemoglobin (g/dl)	14.8	14.2	14.8	13.4*	12.0*	13.1*	12.9*
Haematocrit (%)	23.4	23.6	23.2	22.8	23.1	29.0*	28.1*
MCV (cubic microns)	54.1	54.2	54.4	54.3	52.7*	53.3	53.2
Reticulocytes (units not stated)	4.90	4.87	4.74	4.44	4.19	3.69*	3.76*
Females (week 13)							
Red blood cells (millions)	4.32	4.25	3.92	3.74*	4.18	3.46*	3.72*
Haemoglobin (g/dl)	14.5	14.0	13.2*	14.9	15.5*	15.3	14.8
Haematocrit (%)	22.0	21.6	19.1*	20.3	23.1	18.3*	20.2
MCV (cubic microns)	50.7	50.5	48.2*	53.9*	54.9*	52.3	53.9*
Reticulocytes (units not stated)	3.85	3.69	4.01	4.25*	4.14	4.70*	4.45*

The results of the male and female haematological evaluations are contradictory. In males, there were significant increases in the mean red blood cell counts and haematocrits at 600 ppm and above, the haemoglobin was significantly reduced at 300 ppm and above and reticulocyte counts were significantly reduced at 600 ppm and above. In females, the red blood cell counts were lower than control values and the reticulocyte counts were increased at 300 ppm and above. Male mice appear to have compensated for the decreased haemoglobin levels by increasing the number of circulation red blood cells. The changes in the red blood cell parameters in female mice may be indicative of the beginning of the compensation mechanisms.

There were no compound-related effects on clinical chemistry or urinalysis parameters.

At necropsy, males had increased absolute and relative liver weights at 480 ppm and above. Other organ weight differences were considered due to minor differences in final body weights and not test substance-related. There were no consistent test substance-related gross or microscopic pathology findings noted.

## Conclusions

Under the conditions of this study, the NOAEL in male mice was 150 ppm (approximately 26.6 mg/kg bw/day) based on haematology changes at 300 ppm and above. The NOAEL in female mice was 75 ppm (approximately 15.6 mg/kg bw/day) based on haematology changes at 150 ppm and above. No cholinesterase determinations were performed during this study.

(Cox, 1979b)

b)

<b>Report</b>	Cox, R.H. (1979). Oncogenicity/chronic toxicity evaluation of Nudrin® in B6C3F1 mice for Shell Oil Company. Unpublished DuPont Report No.: TPS 52G-005-650-79. (in-life phase: 1979).
<b>Test facility</b>	Toxicology and Pathology Services, Inc., P.O. Box 333, Mt. Vernon, Indiana 47820 USA.
<b>Guidelines</b>	Based on company protocol.
<b>Deviations from guidelines</b>	The study was terminated at 23 weeks (no longer required for registration purposes).
<b>GLP</b>	No
<b>Test substance</b>	DPX-X1179 Technical.
<b>Batch</b>	SD 14999-Tech
<b>Purity</b>	Not specified
<b>Appearance</b>	White solid
<b>Acceptable</b>	Not acceptable for regulatory purposes on a stand-alone basis (but contains relevant scientific information).

### Materials and methods

Groups of B<sub>6</sub>C<sub>3</sub>F<sub>1</sub> mice were administered diet containing methomyl at concentrations of 0 (160/sex), 100 (80/sex), 300 (80/sex), 1200 (79 males and 81 females) and 1800 ppm (79 males and 81 females) for 23 weeks. Because of the premature termination of this study and the protocol amendments, the report did not contain the details or the results that would be expected in a standard carcinogenicity study.

Clinical observations were carried out at least twice daily. Each animal received a general examination weekly (for palpable masses and abdominal distension). Body weights and food consumption were recorded weekly for the first 13 weeks and then every two weeks thereafter. No clinical chemistry or urinalysis results were included in the report. Haematological parameters were evaluated at 23 weeks. At necropsy (23 weeks), 30 control/sex and 15/sex/test group were fasted sacrificed overnight and then killed by exposure to chloroform. These animals received a gross examination and selected organs (liver, kidneys and spleen) were removed and weighed. Animals that were found dead or were killed before the scheduled sacrifice were subjected to a gross examination.

### Results

No compound-related mortalities occurred and no clinical signs were observed during this study. An apparent compound-related decrease in body weight gains resulting in smaller group mean body weight values was noted in 1800 ppm males at weeks 7-23. After 23 weeks of treatment, the 1800 ppm males weighed 4% less than the respective controls. Additionally, significantly smaller group mean body weight values were noted for 1200 ppm males at weeks 7, 10, and 12 and for 1800 ppm females at weeks 8 and 10. There were no test substance-related effects on food consumption during the course of the study.

The main haematological changes are presented in Table 6.4. There were significant reductions in the mean RBC counts at 1200 ppm and above in males and at 1800 ppm in females. Significant reductions in the mean haemoglobin levels were seen in both sexes at 1200 ppm and above. Significant reductions in the haematocrit values were seen in males at 300 ppm and above and at 1800 ppm in females. The mean

reticulocyte counts were increased at 1200 and above in males and at 1800 ppm in females.

Table 6.26. Mean haematological changes at 23 weeks (units not provided)

Dose (ppm)	0	100	300	1200	1800
Males					
RBC counts	9.19	9.21	8.91	8.46*	8.54*
Haemoglobin	13.6	13.9	13.5	12.2*	12.2*
Haematocrit	41.2	41.8	36.2*	36.8*	35.5*
Reticulocytes	4.0	3.9	4.4	7.6*	6.0*
Females					
RBC counts	9.22	9.32	9.37	9.05	8.54*
Haemoglobin	13.0	13.4	13.9*	12.2*	11.9*
Haematocrit	40.1	40.9	41.4	39.3	36.6*
Reticulocytes	3.3	4.0	3.6	4.0	7.4*

The main organ weight changes are presented in Table 6.27. Mean relative liver weight was increased at 300 ppm and above in males and at 1200 ppm and above in females. Mean relative spleen weight was increased at 300 ppm and above in males and 1800 ppm and above in females.

Table 6.27. Mean organ weight changes at 23 weeks (absolute and relative to body weight)

Dose (ppm)	0	100	300	1200	1800
Males					
Absolute liver weight (g)	1.37	1.35	1.45	1.45	1.50*
Relative liver weight	4.41	4.66	4.72*	5.02*	5.30*
Absolute spleen weight (g)	0.10	0.09	0.12*	0.11	0.14*
Relative spleen weight	0.32	0.30	0.40*	0.39*	0.51*
Females					
Absolute liver weight (g)	1.11	1.15	1.13	1.27*	1.30*
Relative liver weight	4.56	4.61	4.61	4.96*	5.43*
Absolute spleen weight (g)	0.13	0.12	0.12	0.15*	0.16*
Relative spleen weight	0.52	0.49	0.49	0.59	0.66*

No gross compound-related findings were noted at necropsy.

## Conclusions

Under the conditions of this study, a NOAEL of 100 ppm were determined for haematological changes in males and females (approximately 16.6 mg/kg bw/day and 23.2 mg/kg bw/day, respectively).

(Cox, 1979)

### B.6.3.3 Oral studies in dogs (IIA 5.3.2)

a)

<b>Report</b>	Sherman, H. (1967). Three-month feeding study on dogs with S-methyl N-[(methylcarbamoyl)oxy] thioacetimidate [Lannate® methomyl insecticide; INX-1179]. Unpublished DuPont Report No: HLR 168-67 (in-life phase: 1967).
<b>Test facility</b>	DuPont Haskell Laboratory, P.O. Box 50, Elkton Road, Newark, Delaware, 19714
<b>Guidelines</b>	Directive 87/302/EEC Part B: Subchronic toxicity - nonrodent
<b>Deviations from guidelines</b>	The dogs were older than 6-9 months at the initiation of dosing. An ophthalmology exam was not conducted. The following haematology/clinical chemistry parameters were missing: a measure of clotting time and electrolyte balance. The following tissues were not collected/evaluated: jejunum, ileum, rectum and lymph node.
<b>GLP</b>	No
<b>Test material</b>	DPX-X1179
<b>Batch No</b>	X1179-68
<b>Purity</b>	97.5%
<b>Appearance</b>	White crystalline powder
<b>Acceptable</b>	Not acceptable for regulatory purposes on a stand alone basis (but contains relevant scientific information).

#### Materials and methods

Beagle dogs aged 11-13 months (4 dogs/sex/group) were administered methomyl (purity 97.5%) at concentrations of 0, 50, 100, and 400 ppm in the diet for 3 months.

Body weight and food consumption were recorded weekly. Haematology and clinical chemistry parameters were measured and urinalysis were performed pre-test and after 1, 2 and 3 months. At termination the dogs were sacrificed by electrocution and subjected to gross examination. Selected organs and tissues were removed and weighed. A comprehensive list of organs and tissues were preserved in Bouin's fluid and stained with Haskell quadrichrome and examined microscopically.

#### Results

The diets were prepared fresh each week but no analytical results were included in the report. The mean daily intakes of test material are presented in Table 6.28.

Table 6.28. Mean daily intakes of methomyl in males and female dogs

Dose (ppm)	Daily intakes (mg/kg bw/day)	
	Males	Females
50	1.44	1.45
100	3.18	3.01
400	14.68	12.5

There were no deaths or clinical signs of systemic toxicity during the study. Body weight and food consumption were not affected by treatment. No compound-related effects were detected by the haematological and clinical chemistry evaluations or by urinalysis. There were no compound-related gross pathological findings or organ weight changes. No gross or microscopic findings were noted that could be attributed to administration of the test substance

## Conclusion

Under the conditions of this study, the NOAEL was 400 ppm for males and females (14.7 and 12.5 mg/kg bw/day for males and females, respectively), the highest dose tested. No cholinesterase determinations were performed during this study.

(Sherman, 1967).

b)

<b>Report</b>	Busey, W.M. (1968). Two-year dietary administration in dogs: Lannate methomyl insecticide (S-methyl-N-((methylcarbamoyl)oxy) thioacetimidate). Unpublished DuPont Report No.: Hazleton 201-165 and accompanying Addendum No. 1. (in-life phase: March 1966 to March 1968).
<b>Test facility</b>	Hazleton Laboratories America, Inc., 9200 Leesburg Turnpike, Vienna, Virginia, 22580 USA.
<b>Guidelines</b>	Directive 87/302/EEC Part B
<b>Deviations from guidelines</b>	± 20% of the mean weight variation on test day 0 was not determined. Quarantine period was not specified in the report. Some haematology/clinical chemistry parameters were not evaluated: urine volume. The following tissues were not collected/evaluated: parathyroid, trachea, aorta, salivary glands, oesophagus, caecum, rectum, accessory genital organs, mammary gland and femur.
<b>GLP</b>	No
<b>Test material</b>	DPX-X1179
<b>Batch No</b>	X1179
<b>Purity</b>	100 % (weeks 1-60) and 90% (weeks 61-104).
<b>Appearance</b>	White crystalline material.
<b>Acceptable</b>	Not acceptable for regulatory purposes on a stand-alone basis (but contains relevant scientific information).

## Materials and methods

Groups of Beagle dogs (4 dogs/sex/group) were administered methomyl (purity 100, 90%) in their diet at concentrations of 0, 50, 100, 400 or 1000 ppm for 2 years. One dog/sex from each of these dose groups was sacrificed after one year on the study.

Body weight and food consumption were recorded weekly. Clinical signs were recorded daily. Haematological and clinical chemistry investigations and urinalysis were carried out pre-test, 3, 6, 12, 18 and 24 months. Plasma and red blood cell cholinesterase activity were evaluated at pre-test, at 9 weeks (control and 1000 ppm groups) and at 13 weeks (1000 ppm group only). A manometric method was used to determine cholinesterase activity (no further details provided). All dogs received a gross necropsy and selected organs were removed and weighed (brain, heart, liver, spleen, kidneys, adrenals and thyroid). A range of tissues and organs were preserved for microscopic examination and bone marrow smears were taken from the sternum. Only selected tissues were examined microscopically at the 12-month sacrifice.

## Results

Analysis of the diet for concentration, homogeneity and stability was not reported. Statistical analysis of the individual animal data was not always included in the report and the means of the terminal body weights included the body weights of the interim animals.

One 1000 ppm female was found dead during week 9. This dog was replaced on study and the replacement dog also died after 18 days on test. Death of the replacement dog was preceded by convulsive seizures and coma. The deaths of these 2 females were considered test substance-related. A third solitary female dog was then administered 1000 ppm of the test substance using the same experimental regimen in a separate study (Addendum 1).

At 1000 ppm, two males exhibited tremors, salivation, incoordination, and circling movements during the thirteenth week of the study. There were no treatment-related effects on bodyweight or food consumption.

Slight to moderate anaemia was observed in four 1000 ppm dogs at the 3-month interval and persistent treatment-related anaemia was evident in one 1000 ppm male. In the case of the dog with persistent anaemia, the reticulocyte count was increased (30%) and the platelet count was low (172,000/cmm) at 18 months. Withdrawal of the test substance over 10 consecutive weeks (weeks 85-94) from this dog resulted in improvement of the condition but the adverse effects returned on the resumption of dosing. Moderate leukopenia was evident at 1000 ppm in some animals. The main haematological changes are presented in Tables 6.29 and 6.30.

Table 6.29. The main haematological changes (mean values) in dogs at 3 months (n = 4)

Dose (ppm)	0	50	100	400	1000
Males (n = 4)					
RBC (x 10 <sup>6</sup> /cmm)	7.4	6.6	6.3	6.2	4.61
Haemoglobin (g %)	17.3	16.2	16.6	16.6	12.1
Haematocrit (%)	47.4	43.8	46.4	47.9	35.8
Females (n = 4)					
RBC (x 10 <sup>6</sup> /cmm)	7.0	6.5	6.5	7.1	5.4
Haemoglobin (g %)	17.0	16.3	16.9	17.3	13.6
Haematocrit (%)	46.5	45.3	46.4	49.6	40.9

Key: a) RBC =red blood cells.

Table 6.30. Haematological changes in the 1000 ppm dog that exhibited persistent anaemia (no dosing weeks 85-94).

Months	0	3	6	12	18	19	20	21	22	24
RBC (x 10 <sup>6</sup> /cmm)	6.57	4.59	3.54	2.10	2.0	2.8	5.0	5.3	4.5	2.5
Haemoglobin (g %)	14.4	12.1	8.8	6.7	5.1	8.0	10.4	11.4	9.0	6.8
Haematocrit (%)	44	37	28.5	21.5	16.3	23.8	29.7	32.5	26.5	21.0

Key: a) RBC =red blood cells. b) Where repeat evaluations have been made at the same time point the mean of the repeat values is given.

There were no treatment-related effects on the clinical chemistry or urinalysis parameters. The plasma and red cholinesterase determinations are presented in Table 6.31.



Table 6.31. The mean cholinesterase determinations in the 2-year dog study (no further data for any other time points)

Dose (ppm)	0	50	100	400	1000
Males (n = 4)					
<sup>a</sup> Plasma cholinesterase					
0 months	39.6 (5.6)	48.2 (7.1)	47.7 (1.8)	48.7 (3.5)	44.6 (5.6)
2 months	37.3 (5.1)	<sup>b</sup> -	-	-	41.2 (1.3)
3 months	-	-	-	-	36.6 (4.0)
<sup>a</sup> RBCcholinesterase					
0 months	9.0 (2.1)	10.9 (0.96)	11.9 (3.3)	12.4 (2.1)	10.2 (2.2)
2 months	10.0 (1.7)	-	-	-	9.0 (0.66)
3 months	-	-	-	-	15.2 (5.8)
Females (n = 4)					
<sup>a</sup> Plasma cholinesterase					
0 months	41.7 (5.5)	46.6 (9.2)	52.2 (3.6)	44.8 (3.1)	<sup>c</sup> 45.8 (5.3)
2 months	36.0 (4.8)	-	-	-	<sup>c</sup> 41.0 (1.3)
3 months	-	-	-	-	<sup>c</sup> 39.5 (9.9)
<sup>a</sup> RBCcholinesterase					
0 months	12.5 (2.7)	13.1 (3.5)	12.1 (2.2)	9.4 (0.90)	<sup>c</sup> 11.1 (2.7)
2 months	11.8 (0.95)	-	-	-	<sup>c</sup> 10.6 (1.8)
3 months	-	-	-	-	<sup>c</sup> 13.0 (1.7)

Key: a) No units specified or further information on the methodology of these determinations. b) Not evaluated. c) n = 3.

Brain cholinesterase activity was not determined at termination. Plasma cholinesterase activity was slightly lower than the control value in one male dog and one female dog administered 1000 ppm.

At necropsy, the gross findings in the two 1000 ppm female decedents included enlarged liver with white discoloration and dark coloured organ surfaces (lungs, kidneys, spleen). Additional findings included froth in the trachea and bronchi of the lungs or collapsed lungs, scattered diffuse red areas in the pancreas, reddening and scattered petechiae in the gastrointestinal tract and sub-endocardial haemorrhages in the left ventricle of the heart.

The terminal body weights and organ weights are presented in Tables 6.32 and 6.33.

Table 6.32. Mean organs weights (absolute and relative to body weight) and mean terminal body weights at 24 months in male dogs

Dose (ppm)	0	50	100	400	1000
<b>Male terminal sacrifice (n = 3)</b>					
Mean terminal body weight (kg)	8.6 (0.67)	9.5 (1.2)	9.3 (0.66)	8.2 (0.12)	7.5 (0.76) (13%↓) <sup>a</sup>
Absolute liver weight (g)	258.7 (49.6)	240.0 (25.8)	281.7 (39.3)	272.3 (7.0)	293.3 <sup>b</sup> (93.98) (13%↑) <sup>a</sup>
Relative liver weight (org/bw x 100)	2.9871 (0.3656)	2.5409 (0.0765)	3.0044 (0.2431)	3.352 (0.0929) (12%↑) <sup>a</sup>	3.9831 <sup>b</sup> (1.4661) (33%↑) <sup>a</sup>
Absolute spleen weight (g)	23.7 (4.0)	23.1 (3.9)	20.97 (2.3)	26.8 (4.4) (13%↑) <sup>a</sup>	29.8 <sup>c</sup> (2.6) (26%↑) <sup>a</sup>
Relative spleen weight (org/bw x 100)	0.2737 (0.0242)	0.2443 (0.0300)	0.2258 (0.0294)	0.3290 (0.0586) (20%↑) <sup>a</sup>	0.3929 <sup>c</sup> (0.0767) (44%↑) <sup>a</sup>
Absolute kidney weight (g)	49.57 (5.7)	52.8 (5.4)	53.97 (5.5)	55.1 (10.3) (11%↑) <sup>a</sup>	59.5 (4.6) (20%↑) <sup>a</sup>
Relative kidney weight (org/bw x 100)	0.5293 (0.0390)	0.5596 (0.0371)	0.5771 (0.0220) (9%↑) <sup>a</sup>	0.6764 (0.1335) (28%↑) <sup>a</sup>	0.7956 (0.0805) (50%↑) <sup>a</sup>

Key: a) Change compared to control values. b) The dog with persistent anaemia had a liver weight of 426 g. c) n = 2 (the spleen of the dog with persistent anaemia was not included because of its 'extraordinary size').

At 1000 ppm, the mean terminal body weight of male and female dogs was noticeably reduced at 1000 ppm. In males, there were significant dose related increases in mean liver, kidney and spleen weight at 400 ppm and above. Although no weights were provided, the report states that one dog in each of the 100 ppm, 400 ppm and 1000 ppm dose groups had 'rather large prostate glands' (but no microscopic changes).

In females, there was a dose-related increase in relative spleen weight at 400 ppm and above and an increase in relative kidney weight at 1000 ppm. There was a significant reduction in mean absolute liver weight (n = 2) in 1000 ppm females and the relative liver weight is comparable to the control value.

Table 6.33. Mean organs weights (absolute and relative to body weight) and mean terminal body weights at 24 months in female dogs

Dose (ppm)	0	50	100	400	1000
<b>Female terminal sacrifice (n = 3 at ≤ 400 ppm &amp; n = 2 at 1000 ppm)</b>					
Mean terminal body weight (kg)	7.7 (1.6)	9.6 (1.9)	7.2 (0.62)	7.2 (0.68)	5.6 (0.55) (27%↓) <sup>a</sup>
Absolute liver weight (g)	236.7 (45.0)	255.3 (37.68)	220.7 (25.2)	223.3 (44.1)	163 (18.0) (31%↓) <sup>a</sup>
Relative liver weight (org/bw x 100)	3.0838 (0.1629)	2.6740 (0.1385)	3.0714 (0.0939)	3.0959 (0.4415)	2.9340 (0.0336)
Absolute spleen weight (g)	20.6 (6.0)	22.5 (0.79)	19.7 (4.9)	22.6 (3.6)	20.7 (0.15)
Relative spleen weight (org/bw x 100)	0.2812 (0.1027)	0.2410 (0.0410)	0.2722 (0.0547)	0.3169 (0.0367) (13%↑)	0.3755 (0.0346) (33%↑)
Absolute kidney weight (g)	41.5 (7.5)	53.8 (9.6)	40.9 (7.4)	37.97 (6.0)	35.5 (1.35)
Relative kidney weight (org/bw x 100)	0.5411 (0.0132)	0.5612 (0.0461)	0.5683 (0.0716)	0.5273 (0.0462)	0.6244 (0.0376) (15%↑)

Key: a) Change compared to control values.

The microscopic examinations of the interim dogs revealed a pale yellow-brown liver in the 1000 ppm male. At termination, histopathological changes were seen in the spleens and kidneys of the 400 and 1000 ppm male and female groups and in the livers and bone marrow of the 1000 ppm male and female groups. The changes seen in the kidneys were characterised by an increase in pigmentation and slight swelling of the epithelial cells of the proximal convoluted tubules. Pigment deposition was seen in the 400 and 1000 ppm males but only at 1000 ppm in females. Increased pigment and extramedullary haematopoiesis were observed in the spleens of 1000 ppm animals, and pigment was observed in the spleens of 400 ppm animals. Minimal to slight bile duct proliferation in the liver and slightly increased erythroid and myeloid cells in the bone marrow were observed in 1000 ppm animals.

## Conclusions

Under the conditions of this study, the NOAEL was 100 ppm for males and female dogs (3.0 mg/kg bw/day) based on the organ weight and histopathology changes in the kidney and spleen at 400 ppm and above. However, it should be noted that typical cholinergic effects were seen in 1000 ppm males (tremors, salivation, incoordination and circling movements) and 1000 ppm females (convulsive seizures, coma and deaths) but there were no apparent effects on plasma or red blood cell cholinesterase activity. Brain cholinesterase activity was not apparently evaluated (tissue samples, including brain, appear to have been collected and frozen but not analysed).

(Busey, 1968)

### B.6.3.4 Subacute dermal studies on rats (IIA 5.3.3)

Two 21-day dermal toxicity studies have been conducted in rabbits with methomyl.

a)

<b>Report</b>	Brock, W.J. (1989). Repeated dose dermal toxicity: 21-day study with DPX-X1179-394 (methomyl) in rabbits. Unpublished DuPont Report No: HLR 387-89 (in-life phase: April 1989-May 1989).
<b>Test facility</b>	DuPont Haskell Laboratory, P.O. Box 50, Elkton Road, Newark, Delaware, 19714 USA.
<b>Guidelines</b>	Directive 92/69/EEC Method B.9.
<b>Deviations from guidelines</b>	None.
<b>GLP</b>	Yes (inspected by the by the U.S. EPA).
<b>Test material</b>	DPX-X1179
<b>Batch No</b>	X1179-394
<b>Purity</b>	98.4 %
<b>Appearance</b>	White crystalline powder
<b>Acceptable</b>	Yes

#### Material and methods

Groups of New Zealand White rabbits were administered dermal applications of methomyl (purity 98.4%) at dose levels of 0, 5, 50 and 500 mg/kg bw/day (5 or 10/sex/dose) for 21 consecutive days. Dose selection was based on a preliminary range finding studies. The dosing regimen is presented in Table 6.29.

Table 6.34. Dosing regimen

Dose (mg/kg bw/day)	Number of males	Number of females
0	10 <sup>a</sup>	10 <sup>a</sup>
5	5	5
50	5	5
500	10 <sup>a</sup>	10 <sup>a</sup>

Key: a) 5 animals designated to a 14-day recovery group.

On the day prior to study initiation, the dorsal hair (scapular to lumbar region) was closely clipped and the animals were fitted with plastic collars to prevent ingestion and disruption of the dressings. The test substance, moistened with approximately 5 ml deionised water, was applied to an approximate 190 cm<sup>2</sup> area of skin under an occlusive dressing for 6 hours. Approximately 6 hours after treatment, the dressings were removed. The test site was washed with warm water to remove the test substance and the skin was patted dry. One hour later, the site was observed for signs of dermal irritation by the Draize (1959) method.

Prior to each treatment, the rabbits were examined for clinical sign of systemic toxicity and local dermal irritation. Body weights were collected twice per week during treatment and weekly during recovery. Food consumption was determined weekly. Approximately one hour after the last treatment (after removal of the dressings), blood samples were collected from the auricular artery of each rabbit. Five male and 5 female control rabbits and 5 male and 4 female rabbits dosed at 500 mg/kg bw had blood collected 14 days after the last treatment. Plasma, whole blood and brain cholinesterase activities were evaluated at one hour after the last treatment and after

the 14-day recovery period. Blood samples for cholinesterase determinations were stored on ice and assayed as quickly as possible (usually within 30 minutes). A clinical chemistry analyser (Baker Encore) and commercially available reagents (Boehringer Mannheim Diagnostics) were used to determine cholinesterase activities (no further details). Red blood cell cholinesterase activity was calculated from the plasma and whole blood cholinesterase results and the haematocrit.

The animals were sacrificed using barbiturate anaesthesia and exsanguination and subjected to gross examination. At necropsy, the brain was removed and one half was immediately weighed and frozen at -70°C for later analysis. Selected organs were removed and weighed. A comprehensive list of organs and tissues and all gross lesions were fixed for microscopic examination. The organs and tissues and gross lesions from the control and high groups and the treated skin, untreated skin, liver, kidneys, brain and gross lesions from the low- and mid-dose groups were examined microscopically.

## Results

Dosing preparations were prepared daily. Since the dose was quantitatively transferred to a gauze pad and then applied to the application site, verification of the concentration and homogeneity was considered unnecessary. A previous study confirmed that methomyl was stable in water for up to 30 days.

The results of the cholinesterase determinations for the range finding studies are presented in Table 6.35.

Table 6.35. Cholinesterase activities in rabbits treated with 2-500 mg/kg bw/day methomyl for 5 consecutive days (methods as in main study)

Dose (mg/kg bw/day)	Plasma (IU/l)	Red cell (IU/l) <sup>a</sup>	Brain (IU/g)
First range finding study			
Pre-test (n = 4)	410 ± 44	2311 ± 304	-
5 (n = 1)	334 (day 3)	1008 (day 3)	-
	403 (day 5)	3545 (day 5)	6.52 (day 5)
50 (n = 1)	168 (day 3)	1357 (day 3)	-
	239 (day 5)	3620 (day 5)	5.37 (day 5)
200 (n = 1)	276 (day 3)	827 (day 3)	-
	252 (day 5)	2579 (day 5)	4.23 (day 5)
500 (n = 1)	267 (day 3)	1507 (day 3)	-
	168 (day 5)	3068 (day 5)	5.47 (day 5)
Second range finding study			
Pre-test (n = 4)	409 ± 47	3183 ± 275	-
2 (n = 1)	383 (day 3)	3347 (day 3)	-
	416 (day 5)	3580 (day 5)	<sup>b</sup> 7.73 (day 5)
5 (n = 1)	315 (day 3)	2753 (day 3)	-
	340 (day 5)	2858 (day 5)	7.18 (day 5)
25 (n = 1)	366 (day 3)	2980 (day 3)	-
	411 (day 5)	2801 (day 5)	7.16 (day 5)
50 (n = 1)	292 (day 3)	2557 (day 3)	-
	270 (day 5)	3039 (day 5)	6.79

Key: a) Whole blood. b) Mean of left and right brain activity.

In the first range finding study, plasma cholinesterase inhibition was evident at dose levels  $\geq 5$  mg/kg bw/day on day 3 and at dose levels  $\geq 50$  mg/kg bw/day on day 5. Red blood cell cholinesterase inhibition was evident at all dose levels on day 3 but not on day 5. Brain cholinesterase inhibition was 17-35% at dose levels  $\geq 50$  mg/kg bw/day when compared to the brain activity at 5 mg/kg bw/day.

In the second range finding study, plasma cholinesterase inhibition was evident at 50 mg/kg bw/day on days 3 and 5. Red blood cholinesterase activity was not affected by treatment. Brain cholinesterase inhibition was approximately 12% at 50 mg/kg bw/day when compared to the brain activity at 2 mg/kg bw/day.

In the main study, one female was found dead in the top dose group (day 5). The cause of death was 'related to a fracture at the thoracic-cervical junction of the vertebral column'. Microscopically, there was a slight degree of necrosis in the liver and focal haemorrhage in the lungs and thymus. The report considered this death to be traumatic in origin and unrelated to treatment. A significant increase in the incidence of hyperactivity was noted in males at 500 mg/kg bw/day. The clinical observations are presented in Table 6.36.

Table 6.36 Clinical observations

Dose (mg/kg bw/day)	0	5	50	500
Hyperactivity in males	3/10 (30%)	3/5 (60%)	3/5 (60%)	9/10* (90%)
Hyperactivity in females	7/10 (70%)	5/5 (100%)	4/5 (80%)	9/10 <sup>a</sup> (90%)

Key: a) one female found dead on day 5.

There were no effects on body weight, food consumption or food efficiency. The haematological and clinical chemical changes are presented in Table 6.37.

Table 6.37 Mean haematological and clinical chemistry changes (standard deviation)

Dose (mg/kg bw/day)	0	5	50	500
Males (day 21) [n = 5]				
Glucose (mg/dl)	136 (7)	150* (10)	<sup>a</sup> 146 (9)	146* (9)
Males (recovery group/day 35) [n = 5]				
MCHC (g/dl)	30 (0)	-	-	31* (1)
Glucose (mg/dl)	124 (6)	-	-	129 (6)
Females (day 21) [n = 5]				
Platelets ( $\times 10^3$ )	537 (407)	396 (198)	296* (119)	400 (56)
Females (recovery group/day 35)				
No animals	[n = 5]	-	-	[n = 4]
MCV (fl)	64 (2)	-	-	68* (2)
MCH (pg)	19 (1)	-	-	21* (1)
MCHC (g/dl)	30 (0)	-	-	31* (0)
ALT	24 (3)	-	-	30*(4)

Key: a) No indication of statistical significance in report table.

The mean cholinesterase activities are presented in Table 6.38.

Table 6.38. Mean cholinesterase activities

<b>Dose (mg/kg bw/day)</b>	<b>0</b>	<b>5</b>	<b>50</b>	<b>500</b>
<b>Males (day 21) [n = 5]</b>				
Plasma (U/l)	482 (92)	483 (65)	369 (26) [23% ↓]	176 (51) [63% ↓]
Red blood cells (U/l)	2553 (408)	2690 (222)	2446 (394)	2050 (565) [20% ↓]
Brain (U/g)	6.83 (0.66)	6.38 (0.81)	6.18 (0.75) [10% ↓]	3.30 (0.77) [52% ↓]
<b>Males (recovery group/day 35) [n = 5]</b>				
Plasma (U/l)	497 (108)	-	-	441 (33) [11% ↓]
Red blood cells (U/l)	2590 (269)	-	-	2673 (258)
Brain (U/g)	7.05 (0.71)	-	-	6.14 (0.78) [13% ↓]
<b>Females (day 21) [n = 5]</b>				
Plasma (U/l)	461 (84)	489 (104)	413 (58) [10% ↓]	252 (84) [52% ↓]
Red blood cells (U/l)	2801 (503)	3350 (450)	2879 (423)	2380 (346) [15% ↓]
Brain (U/g)	6.91 (0.28)	6.89 (0.45)	5.95 (0.72) [14% ↓]	4.73 (1.10) [32% ↓]
<b>Females (recovery group/day 35)</b>				
No animals	[n = 5]	-	-	[n = 4]
Plasma (U/l)	479 (81)	-	-	414 (34) [14% ↓]
Red blood cells (U/l)	2572 (472)	-	-	2916 (342)
Brain (U/g)	6.47 (0.32)	-	-	6.05 (0.53) [6% ↓]

Gross and microscopic observations and organ weight changes at necropsy were considered incidental and unrelated to test substance administration.

### Conclusions

The NOAEL was determined to be 50 mg/kg bw/day for male and female rabbits. This NOAEL was based on the brain cholinesterase inhibition, effects on the red blood cells and clinical signs at 500 mg/kg bw/day.

(Brock, 1989)

b)

<b>Report</b>	Finlay, C (1997). Methomyl technical: 21-day repeated dose dermal toxicity study in rabbits. Unpublished DuPont Report No: HL-1997-00913 (in-life phase: August 1997 to September 1997).
<b>Test facility</b>	DuPont Haskell Laboratory, P.O. Box 50, Elkton Road, Newark, Delaware, 19714 USA.
<b>Guidelines</b>	Directive 92/69/EEC Method B.9.
<b>Deviations from guidelines</b>	Tissues were not evaluated microscopically. Haematology and clinical chemistry parameters except cholinesterase were not evaluated.
<b>GLP</b>	Yes (inspected by the by the U.S. EPA).
<b>Test material</b>	DPX-X1179
<b>Batch No</b>	X1179-512
<b>Purity</b>	98.6 %
<b>Appearance</b>	White crystalline powder
<b>Acceptable</b>	Yes

### Materials and methods

Groups of New Zealand White rabbits were administered dermal applications of methomyl mixed with distilled water at dose levels of 0, 15, 30, 45 or 90 mg/kg bw/day (6/sex/dose) for 21 consecutive days. Dose selection was based on the previous study. A pilot study was conducted to compare the sensitivity of the cholinesterase assay used in this study with the cholinesterase assay used in the previous study. Blood (0.5 ml) was collected from the jugular vein of 4 male animals to assess the baseline for plasma and red blood cell cholinesterase activity. Then each rabbit was dosed with a single dermal application of 0, 100, 250 or 500 mg/kg bw.

On the day prior to study initiation, the dorsal hair (scapular to lumbar region) was closely clipped and the animals were fitted with plastic collars to prevent ingestion and disruption of the dressings. The test substance, moistened with approximately 5 ml deionised water, was applied to an approximate 190 cm<sup>2</sup> area of shaved skin under a semi-occlusive dressing for 6 hours. Control rabbits were similarly treated with 1 ml deionised water only. Approximately 6 hours after treatment, the dressings were removed. The test site was washed with warm water to remove the test substance and the skin was patted dry. Before treatment each day and after test substance removal, the site was observed for signs of dermal irritation by the Draize (1959) method.

Before treatment and after removal of the test material, the animals were observed for clinical signs (especially typical cholinergic effects), dermal and ocular irritation, abnormal behaviour and mortality. Body weights were recorded twice per week during treatment. Food consumption was determined weekly. Blood was collected from the jugular vein of all rabbits 1 hour following the last treatment (after unwrapping). Plasma and red blood cell cholinesterase activity was evaluated using a modified Ellman method (Boehringer Mannheim/Hitachi 717 clinical chemistry analyser and Boehringer Mannheim Reagents). All rabbits were killed after blood collection on day 21. Following a gross evaluation, brains were collected and weighed, half the brain was frozen (-70°C) and analysed for brain cholinesterase activity (approximately 3 days later).



## Results

Dosing preparations were prepared daily. Since the dose was quantitatively transferred to a gauze pad and then applied to the application site, verification of the concentration and homogeneity was considered unnecessary. A previous study confirmed that methomyl was stable in water for up to 30 days.

There were no mortalities during the study. No significant differences in body weights were observed. There were no test substance-related effects on food consumption or food efficiency. The main clinical signs are presented in Table 6.39.

Table 6.39. Potential compound-induced clinical observations prior to and after dosing

Dose (mg/kg bw/day)	0	15	30	45	90
Males prior to daily dosing					
Pallor eyes	-	-	-	1/6 (day 6)	-
Males after daily dosing					
Ocular discharge	-	-	-	<sup>a</sup> 1/6 (days 14 & 19)	-
Pupillary constriction (both eyes)	-	-	-	<sup>a</sup> 1/6 (day 15)	-
Shivering	-	-	1/6 (day 1)	1/6 (day 1)	2/6 (both day 1)
Females prior to daily dosing					
Conjunctivitis (left eye)			1/6 (day 9)		
Pupillary constriction (one eye)	-	-	-	-	<sup>b</sup> 1/6 (left eye) (days 6 to 21)
Corneal opacity (both eyes)	-	-	-	-	<sup>b</sup> 1/6 (days 5 to-21)
Females after daily dosing					
Pupillary constriction (one eye)	-	-	-	-	2/6* (day 2 in right eye of 1 animal & on day 3 <sup>b</sup> and days 6- 21 in the left eye of the other animal)
Corneal opacity (both eyes)					<sup>b</sup> 1/6 (days 4 to 21)

Key: a) The same animal. b) This single animal had pupillary constriction in one eye and corneal opacity in both eyes. Examination by a veterinary ophthalmologist revealed cataracts (both eyes) and blepharitis, conjunctivitis and corneal erosion/ulcer in the left eye.

At 90 mg/kg bw/day, pupillary constriction, a typical cholinergic effect, was observed in two females. In one female, the pupillary constriction (one eye on day 2) was attributed to splashing of wash water into the animal's eye. In the second female, the pupillary constriction (one eye on days 3 and 6 to 21) was attributed to cataracts (both eyes/no pre-test ophthalmological examination) and blepharitis, conjunctivitis and corneal erosion/ulcer in the left eye. The cataracts were considered to be spontaneous and unrelated to treatment (but no historical control data were provided). The corneal erosion/ulcer was attributed to trauma that in the left eye and resulted in blepharitis and conjunctivitis. At 45 mg/kg bw/day, the pupillary constriction in one male (day 15)

was also attributed to splashing of wash water into the animal's eye. The significance of the shivering observed in males at 30 mg/kg bw/day and above is not clear.

The standard haematological and clinical chemistry parameters were not affected by treatment. The mean cholinesterase determinations are presented in Tables 6.40 & 6.41.

Table 6.40. The results of cholinesterase determinations in the pilot study (n = 1 male)

Dose (mg/kg bw/day )	Plasma cholinesterase (U/l)	Red blood cell cholinesterase (U/l)	Brain cholinesterase (U/g)
0	552	1660	13.62
100	516 [6.5% ↓]	2180	14.03
250	415 [25% ↓]	1640	13.84
500	310 [44% ↓]	1800	8.30 [39% ↓]

Table 6.41. Mean cholinesterase inhibition (standard deviation) in the main study (n = 6)

Dose (mg/kg bw/day )	Plasma cholinesterase (U/l)		Red blood cell cholinesterase (U/l)		Brain cholinesterase (U/g)	
	Males	Females	Males	Females	Males	Females
0	611 (115)	614 (91)	2600 (434)	2493 (383)	14.33 (0.98)	13.58 (1.41)
15	603 (79)	635 (167)	1823 (484) [30% ↓]	2197 (375) [12% ↓]	14.16 (0.93) [1% ↓]	13.78 (0.96)
30	594 (45)	526 (110)	2020 (555) [22% ↓]	2563 (411)	12.91 (0.53) *[10% ↓]	13.62 (0.89)
45	570 (98)	580 (125)	2270 (506) [13% ↓]	2450 (243)	12.65 (0.71) *[12% ↓]	12.70 (0.70) [6% ↓]
90	570 (93) [7% ↓]	526 (116) [14% ↓]	1997 (433) [23% ↓]	2233 (423) [10% ↓]	12.84 (0.80) *[10% ↓]	12.73 (1.00) [6% ↓]

In males, the significant decrease in brain cholinesterase activity at 30 mg/kg bw/day and above did not exhibit a dose-response relationship. The male control value appears to be rather high and there was a 3-fold difference in dose with no clear difference between the mean values. The report considered these differences to be indicative of normal biological variation. The incidence of male brain cholinesterase values that were more than 10% below the control value was 1/6, 2/6, 4/6 and 4/6 at 15, 30, 45 and 90 mg/kg bw/day. However, the decrease in brain cholinesterase in both sexes does not exceed the cut-off (i.e.  $\geq 20\%$ ). The decreases in male red blood cell cholinesterase values are greater than 20% at 15, 30 and 90 mg/kg bw/day but not at 45 mg/kg bw/day. Since these mean red blood cell cholinesterase values were not significantly different from controls and they did not exhibit a dose-response relationship, the report stated that these differences represented normal biological variation and were unrelated to treatment. The incidence of male red blood cell cholinesterase values that were more than 10% below the control value was 1/6, 5/6, 4/6, 2/6 and 5/6 at 0, 15, 30, 45 and 90 mg/kg bw/day. This incidence trend and the number of red blood cell cholinesterase values greater than 20% of the control values suggest that that these values represent a treatment related effect or errors in the methodology. It is noteworthy that the female cholinesterase values were not affected by the treatment at any dose level.

No gross findings were observed at necropsy.

### Conclusions

A NOAEL of 90 mg/kg bw/day was determined for this study, the highest dose used. However, the extensive inter-group variation, the equivocal findings of pupillary constriction, the use of methomyl and not the formulation and the group size of six needs to be considered before using this NOAEL to set a dermal AOEL.

#### B.6.3.5 Subacute inhalation studies on rats (IIA 5.3.3)

The applicant claimed that the toxicological properties of this active substance suggest that short-term repeated dose inhalation studies are unnecessary.

#### B.6.3.6 Summary of short-term toxicity

The NOEL/NOAELs and LOELs are summarised in Table 6.81.

The short-term feeding studies have not been conducted to modern protocols or standards. The studies performed in the 1960s used methomyl with a purity of 90-100% while none of the studies performed in the late 1970s specified the purity of the methomyl tested. None of these studies carried out ophthalmological examinations or carried out reliable determinations to evaluate brain cholinesterase activity. Where cholinesterase activities have been determined, the results are considered to be equivocal because of deficiencies in the reporting and/or the methodology. The overall quality of the oral repeat dose studies summarised in this section is below that normally expected and inadequate for use in the setting of reference values. However, several additional studies were performed to assess the degree and reversibility of cholinesterase inhibition (B.6.8.2-6.8.3).

The most sensitive endpoint in these feeding studies appears to be the haematological changes seen in mice (at 150 ppm) and rats (at 270 ppm). In the 2-year dog study, the most sensitive endpoints appear to be the organ weight and histopathological changes in the kidneys and spleen (400 ppm); haematological changes were evident at 1000 ppm.

In the two 90-day feeding studies in the rat, the main compound-induced effects included reductions in the body weight parameters, in nutritional status and changes in the haematology parameters. Other effects included transient tremors (one animal), clonus convulsions with loss of righting reflex (one animal), reduced serum glucose, erythroid hyperplasia in the bone marrow and organ weight changes (spleen, liver and kidneys). Brain cholinesterase activity was not evaluated in these 90-day rat studies. Plasma and red blood cell cholinesterase activities were evaluated in these studies (no effects in one study and equivocal results in the other). In the 35-week study, there were effects on body weight and red blood cell parameters (only females evaluated). Cholinesterase determinations were made in this 35-week study but the analytical techniques and results were considered to be equivocal.

In the 90-day mouse study, changes in the standard haematological parameters were observed and supported the results in the rat studies (some evidence of reversibility). In the 23-week study, there were effects on body weight, haematological changes and organ weight changes. No cholinesterase determinations were made in these mice studies.

No compound-induced effects were observed in the 3-month dog study. No cholinesterase determinations were reported in this dog study. The method of sacrifice and the non-standard histological staining used in this study are questionable. In the 2-year dog study, there were compound-induced effects on the terminal body weight and the red blood cell parameters, the bone marrow, liver, kidneys and spleen. There were a number of typical cholinergic clinical signs (tremors, salivation, inco-ordination and circling movements, seizures, coma and death) but the expected correlation with reduced cholinesterase activity was not demonstrated. This indicates that the analytical techniques and/or methodology used in this study might have been equivocal.

Two 21-day dermal toxicity studies were conducted in rabbits. In the first study, statistically significant decreases in plasma and brain cholinesterase activities, as well as increased incidences of hyper-reactivity, were observed in male and female rabbits at the high dose (500 mg/kg bw/day). In the second study, there were no clear treatment-related effects on plasma, red blood cell or brain cholinesterase activities. Shivering and pupil constriction (not seen in the previous study at a higher dose level) were reported but lacked clear dose-response relationships and/or were considered not to be related to methomyl administration. A NOAEL of 90 mg/kg bw/day was determined for this second study, the highest dose used. However, the extensive inter-group variation, the equivocal findings of pupillary constriction, the use of methomyl and not the formulation and the group size of six needs to be considered before using this NOAEL to set a dermal AOEL.

## B.6.4 Genotoxicity

### B.6.4.1 *In vitro* testing (AII.5.4.1)

#### a) Gene mutation in bacterial cells

<b>Report</b>	Mathison, B.H. (1997). DPX-X1179 (methomyl): Mutagenicity testing in the Salmonella typhimurium and Escherichia coli plate incorporation assay. Unpublished DuPont Report No.: HL-1997-00043. (in-life phase: January 1997).
<b>Test facility</b>	DuPont Haskell Laboratory, P.O. Box 50, Elkton Road, Newark, Delaware, 19714 USA.
<b>Guidelines</b>	OECD 471 and 472 (1983). Directive 92/69/EEC Method B.13. and B.14.
<b>Deviations from guidelines</b>	S. typhimurium: TA 97a was used instead of TA 1537. For E coli, only one strain (WP2uvr A(pKM 101)) was used.
<b>GLP</b>	Yes (inspected by the by the U.S. EPA).
<b>Test substance</b>	DPX-X1179 Technical.
<b>Batch</b>	X1179-394.
<b>Purity</b>	98.4%
<b>Appearance</b>	White solid
<b>Acceptable</b>	Yes

## Materials and Methods

Methomyl (purity 98.4%) was evaluated for mutagenicity in *Salmonella typhimurium* strains TA97a, TA98, TA100, TA1535, and in *Escherichia coli* strain WP2 *uvrA* (pKM101) with and without metabolic activation (Aroclor-induced rat liver S9 fraction). Nominal concentrations of 0, 10, 50, 100, 250, 500, 1000, 2500 and 5000 µg/plate (3 plates) were evaluated using standard plate incorporation methods (Trial 1, tester strains TA100 and *E. coli* only). Based on the lack of evidence of toxicity and the absence of test substance precipitation in an initial evaluation using tester strains TA100 and *E. coli* at concentrations of 10, 50, 100, 500, 1000, 2500, and 5000 µg/plate, subsequent tests were conducted in strains TA97a, TA98, and TA 1535. A second independent test was carried out using the same concentrations and all strains. Appropriate positive and negative control substances were tested.

## Results

The number of revertants at all concentrations of the test substance was similar to concurrent controls in studies both with and without metabolic activation. There was no evidence of test substance precipitate or toxicity. Appropriate results were obtained with the positive control compounds

## Conclusions

The test substance was negative for mutagenic activity in two independent trials.

(Mathison, 1997)

### b) Gene mutation in mammalian cells

<b>Report</b>	McCooley, K.T. (1984). CHO/HGPRT assay for gene mutation. Unpublished DuPont Report No.: HLR 556-83 Revision 1.(in-life phase: 1983)
<b>Test facility</b>	DuPont Haskell Laboratory, P.O. Box 50, Elkton Road, Newark, Delaware, 19714 USA.
<b>Guidelines</b>	Directive 87/302/EEC Part B
<b>Deviations from guidelines</b>	None
<b>GLP</b>	No.
<b>Test substance</b>	DPX-X1179 Technical.
<b>Batch</b>	X1179-255
<b>Purity</b>	99%
<b>Appearance</b>	White solid
<b>Acceptable</b>	Yes

## Materials and Methods

Methomyl was tested in the CHO/HGPRT mutation assay with and without an exogenous metabolic activation system (Aroclor-induced rat liver S9 fraction). The test substance was dissolved in dimethyl sulfoxide (DMSO). Dose selection was based on a preliminary toxicity assay.

Two independent assays were conducted using duplicate flasks of exponentially growing CHO-K<sub>1</sub>-BH<sub>4</sub> cells exposed for 18-19 hours at 37°C to concentrations of 0,

10, 20, 40, 50, and 55 mM in the non-activated system, and for 5 hours to concentrations of 0, 0.1, 0.15, 0.2, 0.25, and 0.35 mM in the S-9 activated system. All assays included appropriate concurrent negative and positive control cultures.

Cells were then independently subcultured for assessment of cytotoxicity (cloning efficiency) and for expression and selection of the 6-thioguanine (TG, 2-amino-6-mercaptopurine)-resistant phenotype. Toxicity was defined as a cloning efficiency of  $\leq 50\%$  of the concurrent vehicle controls. The assay was considered positive when the mutant frequency of one or more of the sample concentrations tested was significantly greater than the solvent control and when the correlation between the mutant frequency and the concentration of the test sample was significantly greater than 0.

## Results

Cytotoxicity was observed at concentrations of 40 mM and above in the non-activated test system, and 0.15- 0.20 mM and above in the activated test system. There were no statistically significant increases of the mutant frequency relative to the solvent control, and there was no significant positive linear dose response. Appropriate results were obtained with the positive control compounds.

## Conclusions

Methomyl was negative in the CHO/HGPRT mutation assay.

(McCooey, 1984)

### c) Mammalian cytogenicity test (clastogenicity)

The company have not conducted an *in vitro* cytogenetics study with methomyl. However, two *in vivo* studies that measure chromosome aberrations and micronuclei in rodent bone marrow have been performed and are summarised in section B.6.42.

### d) Unscheduled DNA synthesis

<b>Report</b>	Vincent, D.R. (1985). Assessment of methomyl (INX-1179-255) in the <i>in vitro</i> unscheduled DNA synthesis assay in primary rat hepatocytes. Unpublished DuPont Report No.: HLR 149-85, Revision 1. (in-life phase: 1983-1985 )
<b>Test facility</b>	DuPont Haskell Laboratory, P.O. Box 50, Elkton Road, Newark, Delaware, 19714 USA.
<b>Guidelines</b>	Directive 87/302/EEC Part B: DNA damage and repair Directive
<b>Deviations from guidelines</b>	Metabolic activation was not used since the study was conducted with fresh hepatocyte cultures.
<b>GLP</b>	No.
<b>Test substance</b>	DPX-X1179 Technical.
<b>Batch</b>	X1179-255
<b>Purity</b>	99%
<b>Appearance</b>	White solid
<b>Acceptable</b>	Yes

## Materials and Methods

Methomyl was tested in the unscheduled DNA synthesis (UDS) assay using primary cultures of rat hepatocytes from male Cr1:CD rats. The concentrations tested in the media were 0, 1, 10, 100, 1000, 5000, 10000 and 75000 µM. The highest dose level was set based on solubility limitations and the need to limit the concentration of the organic solvent to 1% (v/v) in the treatment medium. Appropriate positive and vehicle controls were used. Hepatocyte cultures were exposed to the test substance, along with 5 µCi [<sup>3</sup>H]-thymidine/ml, for approximately 18 hours. Mean net nuclear grain counts (cells with ≥ 5 net nuclear grains (NNG)) were obtained for each group from autoradiography slides. Cytotoxicity was assessed based on lactate dehydrogenase (LDH) concentration in the culture medium and by microscopic examination of hepatocyte cultures and of fixed and stained cells. Four trials were performed, but trials 1 and 2 were rejected based on unacceptable negative and positive control results. Only the results of trials 3 and 4 were presented in the study report.

## Results

The limit of solubility of the test substance in treatment medium was found to be 100 µM. Cytotoxicity, determined by elevation of LDH activity in the medium, was observed at concentrations of 5000 µM and above. No UDS was observed at any concentration of the test substance and statistical analyses indicated neither a compound related effect, nor a dose-response relationship.

## Conclusions

Methomyl did not cause a significant increase in unscheduled DNA synthesis in rat hepatocytes and was concluded to be negative in this assay.

(Vincent, 1985)

### B.6.4.2 *In vivo* genotoxicity in somatic cells (AII 5.4.2)

#### a) Chromosome aberration assay in the rat (metaphase analysis)

<b>Report</b>	Anon. (1984). <i>In vivo</i> bone marrow chromosome study in rats. Unpublished DuPont Report No.: HLO 63-84. (in-life phase: 1983)
<b>Test facility</b>	Hazleton Laboratories America, Inc., 9200 Leesburg Turnpike, Vienna, Virginia, 22580, USA.
<b>Guidelines</b>	Directive 92/69/EEC Method B.11
<b>Deviations from guidelines</b>	None
<b>GLP</b>	No (a signed quality assurance statement is included in the report)
<b>Test substance</b>	DPX-X1179 Technical.
<b>Batch</b>	X1179
<b>Purity</b>	99%
<b>Appearance</b>	White solid
<b>Acceptable</b>	Yes

## Materials and methods

Methomyl was evaluated for its ability to induce clastogenicity (chromosome-breakage) in bone marrow cells of male and female Sprague-Dawley, CD rats (5 animals/sex/group). The animals were sacrificed at each of three time intervals (6, 24,

and 48 hours) following dosing. Both male and female rats (15 animals/sex/dose group) received a single dose of methomyl suspended in distilled water at concentrations of 2, 6, or 20 mg/kg bw by oral gavage. Doses were adjusted to 100% of the target doses based upon a purity of 99% active ingredient. A dosing volume of 5 ml/kg was used. Negative (vehicle) controls were included at all three sacrifice intervals. A single positive control group was treated and sacrificed at the 24-hour interval. Distilled water was used as the negative control and cyclophosphamide (40 mg/kg bw in distilled water) was used as the positive control. Two hours prior to sacrifice, each animal received a single i.p. injection of 2.0 mg colchicine/kg body weight to arrest dividing cells in metaphase.

Clinical signs were assessed for all groups twice daily or before sacrifice. Body weights were recorded once, prior to compound administration, for the 6-hour sacrifice, and twice, prior to compound administration and prior to colchicine administration, for the 24- and 48-hour sacrifices. Metaphase cells from the bone marrow of male and female rats were collected at three time intervals (6, 24, and 48 hours) following dosing. The cells were stained with Giemsa. A total of 500 cells per animal were evaluated to assess toxicity based on the average number of mitoses (mitotic index), and fifty metaphase cells per animal (250 metaphases/sex/dose group) were analysed for structural chromosome aberrations.

## Results

One animal from the 2 mg/kg bw dose group appeared slightly depressed (as stated in report) and one animal from the 6 mg/kg bw dose group was observed to have red stains around nose and/or eyes during the post treatment period. A few animals from the 20 mg/kg bw dose group showed abnormal observations including rough coat, urine stains, slight depression, or red stains around nose and/or eyes. Two animals from the 20 mg/kg bw dose group were found dead on the afternoon of dosing. No significant variance in mean body weight changes was observed in any of the treatment groups in male or female rats.

No statistically significant differences were seen in mitotic indices between the methomyl-treated groups and the concurrent negative controls at any sampling interval. No statistically significant increases in structural or numerical chromosome aberrations were observed at any dose level at any time interval (6, 24 or 48 hours). Appropriate results were observed for the positive and negative control groups.

## Conclusions

Methomyl did not induce structural or numerical chromosome aberrations in bone marrow cells of rats and is considered non-clastogenic under the conditions in this *in vivo* test.

(Anon, 1984)



## b) Mouse bone marrow micronucleus

<b>Report</b>	Bentley, K.S. (1995). Mouse bone marrow micronucleus assay of DPX-X1179-394. Unpublished DuPont Report No.: HLR 413-95 (in-life phase: 1995).
<b>Test facility</b>	DuPont Haskell Laboratory, P.O. Box 50, Elkton Road, Newark, Delaware, 19714 U.S.A.
<b>Guidelines</b>	Directive 92/69/EEC Method B.12 and OECD 474 (1983).
<b>Deviations from guidelines</b>	None
<b>GLP</b>	Yes (inspected by the U.S. EPA)
<b>Test substance</b>	DPX-X1179 Technical.
<b>Batch</b>	X1179-394
<b>Purity</b>	98.4%
<b>Appearance</b>	White solid
<b>Acceptable</b>	Yes

**Materials and methods**

Methomyl was evaluated for its ability to induce micronucleated polychromatic erythrocytes (MNPCEs) in the bone marrow of CR1:CD<sup>-1</sup>(ICR)BR mice at 3 time points. Male and female mice (5 or 6 mice/sex/dose) received a single oral dose (gavage) of 0, 3, 6, or 12 mg/kg bw. The dose volume was 10 ml/kg bw. The highest dose level was set based on a range-finding study conducted up to 40 mg/kg bw. The test substance was suspended in sterile water. Cyclophosphamide (CP, 40 mg/kg bw) was used as the positive control.

In-life observations included clinical signs and body weight determinations. Clinical signs were recorded before dosing, approximately 2.0 to 3.5 hours post-dosing, and daily thereafter. Body weights were recorded prior to dosing and at sacrifice.

Bone marrow smears were prepared approximately 24, 48, and 72 hours after dosing from both male and female animals in all three dose groups. Bone marrow smears were prepared 24 hours after dosing from males and females in the positive control group. Two thousand polychromatic erythrocytes (PCEs) per animal were scored for micronuclei. The number of PCEs per 1000 erythrocytes was also recorded to assess toxic effects on the bone marrow.

**Results**

Two female mice, one in the negative control and one in the 12 mg/kg bw group, died because of improper dosing technique and were removed from the study. One female mouse was found dead approximately 24 hours after dosing at 12 mg/kg bw. This death was considered to be test substance-related. Hyperactivity was displayed in one male mouse at 12 mg/kg bw at the 24-hour observation point. Lethargy was seen in 3 males at 6 mg/kg bw in 3 male mice and in 1 male and 1 female at 12 mg/kg bw 2 to 2.5 hours after dosing. One of these mice had half shut eyes 24 hours post-dosing. Statistically significant mean body weight changes relative to the negative control group occurred in the 12 mg/kg bw-treated male group at the 48-hr sacrifice time point.

There were no statistically significant increases in MNPCE frequency in male or female mice at any sampling time at any dose level tested. In addition, no statistically significant depressions in the proportion of PCEs among 1000 erythrocytes were observed.

### Conclusions

Methomyl did not induce an increase in micronuclei in bone marrow cells of mice and is considered neither a clastogen nor an aneugen in this *in vivo* assay.

(Bentley, 1995)

#### B.6.4.3 *In vivo* studies in germ cells (AII 5.4.3)

Because the results of the standard *in vitro* and *in vivo* mutagenicity assays were negative, no *in vivo* studies in germ cells are required.

#### B.6.4.4 Published studies

Positive results have been reported in the literature but no published studies were submitted for evaluation. The company have evaluated two of these published papers and submitted a summary of their findings.

Two reports pertaining to the potential genotoxic effects of methomyl and an accompanying formulation, Lannate 25 WP (a water soluble powder formulation containing 25% a.s.), appear in the open literature. In the first paper (Bonatti *et al.*, 1994, Environ. Mol. Mut. 23:306-311), the authors report that chromosome aberrations, micronuclei, and DNA damage occurred following a 48-hour incubation of methomyl and Lannate 25 WP in cultures of whole blood lymphocytes *in vitro*. In the second paper (Bolognesi *et al.*, 1994, Environ. Mol. Mut. 24:235-242), the authors report increased frequencies of micronuclei in mouse bone marrow and DNA damage in liver and/or kidney following two intraperitoneal injections of methomyl or Lannate 25 WP at 5 mg as/kg bw. However, the results of these studies are difficult to interpret for a number of reasons:

- The incubation periods *in vitro* were somewhat long (48 hours) and acid generation by hydrolysis of the carbamate moiety of methomyl by endogenous esterases may have artificially produced low pH conditions resulting in DNA and chromosome damage. No assessment of pH is presented (Brusick, D., 1986, Environ. Mut. 8:879-886).
- No clear pattern of the type of chromosome aberrations induced *in vitro* was apparent with dose or test material and no dose-response occurred for DNA damage.
- *In vivo* studies were conducted by a route (i.p. injection) applicable to hazard identification, but not relevant to human dietary or occupational exposure scenarios; whereas, at higher doses given by gavage (12-20 mg/kg bw), no genotoxic effects were produced in two independent studies which assessed chromosome aberration and micronuclei.
- Frequencies of micronuclei were relatively low and within or near the reported historical control ranges (Salamone *et al.*, Environ. Mol. Mut. 23:239-273); no information was given regarding the distribution and frequency of micronuclei in individual animals.

Therefore, the company does not regard these studies as suitable for assessing the genotoxic potential of methomyl.

#### B.6.4.5 Summary of genotoxicity studies

- a) A summary of the company results is presented in Table 6.42.

The company has submitted several studies in which the mutagenic and DNA damaging potential of methomyl has been evaluated in accordance with the protocols of international test guidelines. These studies tested methomyl with a purity of 98.4% or 99%. Negative results were obtained in all of the company studies. Overall, the weight of the evidence from the company's studies and the published literature indicates that methomyl does not pose a mutagenic or genotoxic concern to humans under the proposed conditions of use.

Table 6.42 Summary of the company genotoxicity studies

Study	Concentrations	Result	Reference
<i>In vitro</i> assays			
Ames test	0-5000 µg/plate	Negative	Mathison, 1997.
Mammalian cells (CHO/HGPRT)	0 - 56 mM without S9 0 - 0.35 mM with S9	Negative	McCooley, 1984.
Unscheduled DNA synthesis	0 – 75,000 µM	Negative	Vincent, 1985.
<i>In vivo</i> assays			
Chromosome aberration assay (clastogenicity) in the rat	0, 2, 6 or 20 mg/kg bw	Negative	Anon, 1984.
Bone marrow micronucleus in the mouse	0, 3, 6 or 12 mg/kg bw	Negative	Bentley, 1995.

#### B.6.5 Long-term toxicity and carcinogenicity (AII 5.5)

##### B.6.5.1 2 Year dietary study in rats

<b>Report</b>	Kaplan, A.M. (1981). Long-term feeding study in rats with S-methyl N-((methylcarbamoyl)oxy) thioacetimidate (methomyl; INX-1179). Unpublished DuPont Report No.: HLR 235-81 (5 volumes) (in-life phase: November 1976 to December 1978).
<b>Test facility</b>	DuPont Haskell Laboratory, P.O. Box 50, Elkton Road, Newark, Delaware, 19714 U.S.A.
<b>Guidelines</b>	Directive 87/302/EEC Part B
<b>Deviations from guidelines</b>	± 20% of the mean weight variation on test day 0 was not determined. The following haematology/clinical chemistry parameters were not evaluated: a measure of clotting potential and albumin. Only 10 rats/sex/group were necropsied at 12 months. The following tissues were not collected/evaluated: rectum and femur.
<b>GLP</b>	No
<b>Test substance</b>	DPX-X1179 Technical.
<b>Batch</b>	X1179-255
<b>Purity</b>	99%
<b>Appearance</b>	White solid
<b>Acceptable</b>	Yes

## Materials and methods

Groups of Chr:CD rats (80 rats/sex/group plus an additional 20 rats/sex/group designated for erythrocyte and brain cholinesterase determinations) were administered methomyl in diet at concentrations of 0, 50, 100, and 400 ppm. Ten rats per group were sacrificed after 1 year on study and all surviving rats were sacrificed after approximately 2 years on study. Dose selection was based on a previous 22 month study which revealed histological changes in the kidneys at 400 ppm in both sexes (renal tubule changes) and changes in the spleens of female rats at 200 ppm and above (extramedullary haematopoiesis).

The animals were examined daily for clinical signs of toxicity. Bodyweights and food consumption were recorded weekly for the first six months and every two weeks thereafter. No ophthalmologic examinations were carried out in this study. Haematological and clinical chemistry examinations and urinalysis (10/sex/group) were carried out at 3, 6, 12, 18 and 24 months (the same 10 rats/sex/group were used for all three investigations). Blood samples were collected from the tail vein. Erythrocyte cholinesterase determinations (20 rats/sex/group) were made after 1, 2 and 4 weeks and after 3, 6, 12 and 20 months using the Ellman method (no further details on the analytical techniques or the methodology). Brain cholinesterase determinations were made at 52 weeks (10 designated rats/sex/group/time point) and at 87 weeks (reduced survival in the designated animals).

All rats were subjected to gross examination and selected organs were removed and weighed. Pooled samples of selected tissues were frozen and retained for possible residue analysis (not reported). A comprehensive list of tissues and all gross lesions from each dose level were examined microscopically.

## Results

Analysis confirmed that the concentration, stability and homogeneity of the test material in the diet were acceptable.

There were no compound-related effects on mortality and no compound-related clinical signs of toxicity were noted during the study. At 400 ppm, the mean body weights of males and females were significantly lower during the first year of the study. During the second year, 400 ppm males had a lower mean body weight than controls up to study day 560. There were no significant compound-related effects on food consumption or food efficiency.

The main haematological changes are presented in Table 6.43. At 12 months, there were slight reductions in the female haematocrit and male haemoglobin values at 400 ppm. At termination, females in the 400 ppm group had significantly lower erythrocyte counts, haemoglobin values, and haematocrit when compared to controls. A similar trend occurred in erythrocyte counts and haemoglobin values in male rats fed 400 ppm but these changes were not statistically significant.

Table 6.43. Mean haematological changes at 24-months

Dose (ppm)	0	50	100	400	0	50	100	400
	Males				Females			
Erythrocytes (x 10 <sup>6</sup> /mm <sup>3</sup> )	5.87	5.90	6.06	5.51	5.81	5.64	5.26	5.13*
Haemoglobin (g/dl)	13.9	13.6	14.2	13.3	14.3	14.2	13.4	13.4*
Haematocrit (%)	44	43	44	42	46	45	44	44*

No compound-related clinical chemistry or urinalysis findings were noted.

Erythrocyte cholinesterase activity was slightly lower (not statistically significant) for the 50, 100, and 400 ppm males during the 0.25, 0.5, and 1-month evaluations. However, a dose response-relationship was not evident and there were no effects in females. There were no effects on brain cholinesterase activity at either 52 or 87 weeks.

At the interim sacrifice, there were no compound-related gross findings. The mean relative weight of the 400 ppm male pituitary glands was significantly increased and the mean absolute heart weight of the 400 ppm females was significantly decreased. There were no histological changes in these organs.

No test substance-related gross lesions were observed at necropsy. There were no histopathologic findings noted. There were no test substance-related absolute organ weight changes in either sex at any concentration nor were there any relative organ weight findings in male rats. Female rats at and above 100 ppm had significantly increased relative liver weights; however, this did not correlate with any histopathological changes. Females fed 400 ppm also had increased relative spleen weights, the report suggested that increased spleen weight might correlate with rear footpad dermatitis; a common naturally occurring lesion in wire cage-reared ageing rats but this increased spleen weight is more likely to be related to red blood cell effects. There was no increase in neoplastic lesions in either males or females at any dietary concentration.

## Conclusions

The NOAEL in the 2-year feeding study in rats was 100 ppm for males and females (4.8 mg/kg bw/day for males and 6.3 mg/kg bw/day for females) based on body weight effects and haematology changes at 400 ppm. Under the conditions of this study, methomyl was not carcinogen.

(Kaplan, 1981)

**B.6.5.2 2 Year dietary study in mice**

<b>Report</b>	Snyder, F.G. (1981). 104-week chronic toxicity and carcinogenicity study in mice methomyl; H-11,135. Unpublished DuPont Report No.: HLO 253-81, Final report and accompanying Appendix No. 1. (in-life phase: February 1978 to December 1979).
<b>Test facility</b>	Hazleton Laboratories America Inc., 9200 Leesburg Turnpike, Vienna, Virginia, 22580 USA.
<b>Guidelines</b>	Directive 87/302/EEC Part B
<b>Deviations from guidelines</b>	± 20% of the mean weight variation on test day 0 was not determined. The following tissues were not collected/evaluated: caecum, rectum, femur, spinal cord.
<b>GLP</b>	No
<b>Test substance</b>	DPX-X1179 Technical.
<b>Batch</b>	X1179-262
<b>Purity</b>	99%
<b>Appearance</b>	White solid
<b>Acceptable</b>	Not acceptable for regulatory purposes on a stand-alone basis (but contains relevant scientific information).

**Materials and methods**

Groups of CD-1 mice (approximately 80 mice/sex/group) were administered methomyl in diet at concentrations of 0, 50, 100 (reduced to 75 at week 39) and 800 ppm (reduced to 400 at week 28 and further reduced to 200 at week 39) for 104 weeks.

The animals were examined daily for mortalities and clinical signs of toxicity. Bodyweights and food consumption were recorded weekly for the first six months, then every two weeks to week 52 and every fourth week thereafter. Blood samples (10/sex/group) were collected for haematological determinations from fasted animals by segmental tail amputation during weeks 4, 13, 26, 52, 78 and 104. All animals received a gross examination and selected organs were removed and weighed. A comprehensive list of tissues and organs from each animal were examined microscopically. The head (three coronal sections) and two levels of spinal cord from 10/sex/group were examined microscopically at termination (sections were stained with H & E).

**Results**

The mean daily intakes are presented in Table 6.44.

Table 6.44. Mean daily intakes

<b>Dose (ppm)</b>	<b>Males (mg/kg bw/day)</b>	<b>Females (mg/kg bw/day)</b>
50	8.7	10.6
100/75	15.4	19.1
800/400/200	93.4	118.5

The mortality data are presented in Table 6.45.

Table 6.45. Survival rates at selected time points (denominators have been adjusted at some time points because of missing animals)

Dose (ppm)	0	50	100 <sup>b</sup>	800 <sup>a</sup>	0	50	100 <sup>b</sup>	800 <sup>a</sup>
	Males				Females			
Week 0-5	79/79	80/80	79/79	78/79	80/80	79/80	77/80	80/80
Week 26	78/79	74/80	73/79	69/79	77/80	77/80	70/80	66/80
Week 28	76/77	74/80	73/79	69/79	76/80	76/80	68/80	66/80
Week 38	72/76	70/80	67/79	62/79	73/80	71/80	61/80	56/80
Week 40	72/76	68/80	66/79	62/79	73/80	71/80	61/80	56/80
Week 52	69/76	67/80	64/79	61/79	70/80	70/80	61/80	51/80
Week 68	67/76	61/80	59/79	52/79	64/80	66/80	57/80	49/80
Week 72	67/76	59/80	59/79	52/79	63/80	65/80	53/80	47/80
Week 76	65/76	58/80	56/79	51/79	62/80	62/80	52/80	45/80
Week 80	65/76	57/80	53/79	49/79	60/80	61/80	49/80	43/80
Week 84	64/76	52/80	52/79	47/79	58/80	58/80	48/80	41/80
Week 104	52/76	35/79	30/79	31/79	37/80	40/80	28/80	23/80

Key: a) Reduced to 400 ppm at week 28 and then to 200 ppm at week 39. b) Reduced to 75 ppm at week 39.

Decreased survival occurred in the 100 ppm females and in the 800 ppm males and females. Survival continued to be lower in 75 and 200 ppm males and females and was statistically significantly ( $p < 0.05$  Fisher exact test) lower in all treated males at week 104 and in 800 ppm females. Although survival was reduced, it was  $>50\%$  until week 84 and is not considered to have markedly compromised the potential of the study to detect any carcinogenic response.

No compound-related clinical signs were noted during the study. The incidence of gross or palpable masses was similar for all groups. There were compound-related effects on body weights, food consumption or food efficiency.

The main haematological changes are presented in table 6.46.

Table 6.46. Mean haematological changes at week 26

Dose (ppm)	0	50	100/75	800/400/200
Males				
Haematocrit (%)	51.1	99.2	52.30	47.8
Haemoglobin (g/dl)	16.6	15.7	16.5	14.7
RBC count ( $10^6/\text{mm}^3$ )	9.1	8.8	9.0	8.0
Females				
Haematocrit (%)	51.4	49.8	48.2	50.0
Haemoglobin (g/dl)	16.3	15.7	15.0	15.0
RBC count ( $10^6/\text{mm}^3$ )	8.9	8.7	8.3	8.4

Significantly lower haematocrit was noted in 100 and 800 ppm males at week 13. Significantly lower haemoglobin level and red cell count were noted in 800 ppm males at week 26. Decreases in haemoglobin levels were noted in the 100 and 800 ppm females at weeks 13 and 26 (significantly lower in 100 ppm females at week 13). Decreases in red cell counts were also noted in 100 and 800 ppm females at week 26. These changes are indicative of a test substance-related effect on red cell mass. This trend was not noted after week 26.

There were no compound-related gross or histopathological findings noted. The incidence of neoplastic lesions was similar among all groups. The organ weight data was unremarkable except for a significantly higher absolute and slightly elevated relative adrenal weight in 800 ppm males.

### Conclusions

A NOEL/NOAEL was not proposed for male mice based on the increased mortality rate at 50 ppm, the lowest dose used. An increase in mortality was evident in males at the low dose during weeks 72-104. A NOAEL of 50 ppm was determined for females (equivalent to 10.6 mg/kg bw/day) based on increased mortality at 75 ppm. Under the conditions of this study, methomyl was not a carcinogen.

*The company proposed a NOAEL of 50 ppm for both males and females (8.7 mg/kg bw/day for males and 10.6 mg/kg bw/day for females). This NOAEL was based on increased mortality at 75 ppm and above in males and females.*

(Snyder, 1981)

### B.6.5.3 Summary of chronic toxicity/carcinogenicity

The NOEL/NOAELs and LOELs are summarised in Table 6.81. The methomyl tested in both long-term studies had a purity of 99%.

In the 2-year rat study, body weight effects and mild haematological changes were observed. In the 2-year mouse study, reduced survival and mild transient haematology changes were observed (the haematological changes were not present after 26 weeks).

Methomyl was not carcinogenic in rats or mice.

### B.6.6 Reproductive toxicity

#### B.6.6.1 Multigeneration studies in rats (AII 5.6.1)

Two multigeneration studies have been conducted in rats.

a)

<b>Report</b>	Lu, C.C. (1983). Nudrin 2 generation reproduction study in rats. Unpublished DuPont Report No.: WRC RIR-275 (HLO 519-95) (5 volumes). (in-life phase: March 1979 to March 1980).
<b>Test facility</b>	WIL Research Laboratories, Inc. (USA), Ashland, Ohio, USA.
<b>Guidelines</b>	Directive 87/302/EEC Part B: Two-generation reproduction
<b>Deviations from guidelines</b>	Deviations: In the F <sub>0</sub> generation, 15-21 females/group were pregnant instead of the required 20/group. Litters were standardised to 10 pups instead of 8 pups. In the F <sub>0</sub> generation, the following tissues were not evaluated: cervix, vagina, epididymes, seminal vesicle, coagulating gland, prostate. In the F <sub>1</sub> and F <sub>2</sub> generation, the following tissues were not evaluated: cervix, vagina, epididymes, seminal vesicle, coagulating gland.
<b>GLP</b>	No
<b>Test substance</b>	DPX-X1179 Technical.
<b>Batch</b>	SD 14999-Tech
<b>Purity</b>	Not specified
<b>Appearance</b>	White solid
<b>Acceptable</b>	Not acceptable for regulatory purposes on a stand-alone basis (but contains relevant scientific information).



## Materials and methods

In a multigeneration reproduction study, methomyl was administered in the diet to male and female CD rats at concentrations of 0, 75, 600 or 1200 ppm. Each dose group consisted of 13 male and 26 female rats for the F<sub>0</sub> generation. Due to low conception rates for F<sub>0</sub> rats, additional rats were selected for the F<sub>1</sub> generation at week 12 to increase each group to 20 males and 40 females. The 1200 ppm dose group had 38 females instead of 40 due to a shortage of animals at the time of selection. The F<sub>0</sub> rats were bred within their treatment groups to produce F<sub>1</sub> litters after 100 days on test. The F<sub>1</sub> rats were bred within their respective treatment groups to produce F<sub>2</sub> litters after 120 days on test.

The animals were examined twice daily for mortality and clinical signs of toxicity. Male body weights were recorded weekly. Females body weights were recorded weekly until mating was confirmed, body weights were recorded daily during pregnancy (from day 3 to day 1 postpartum). Male and female food intakes were recorded weekly. Haematology and cholinesterase determinations (no details of analytical techniques or methodology) were conducted in F<sub>0</sub> males and females prior to necropsy. Macroscopic and microscopic examinations were carried out on F<sub>0</sub> and F<sub>1</sub> parental animals at sacrifice (13 males and 26 females/group/generation) and on randomly selected pups (10/sex/group). Selected organs were removed and weighed.

Sperm counts were conducted on the right testis of F<sub>0</sub> and F<sub>1</sub> males. The length of gestation and the fertility index were determined. The litter parameters evaluated included litter size, number of live and stillborn, pup weight, pup sex and clinical observations. On day 4 of lactation, any litters greater than ten pups were randomly culled to ten.

## Results

There were no F<sub>0</sub> or F<sub>1</sub> parental deaths during the study. An increased incidence of alopecia was seen in males and females at 600 ppm and above.

Bodyweight data for the F<sub>0</sub> and F<sub>1</sub> females are presented in Table 6.47.

Table 6.47. Mean body weight values (g) for F0 and F1 females at selected time points

Dose (ppm)	0	75	600	1200
F0 females (weekly recording)				
Week -1	98.8	98.9	99.2	99.2
Week 1	138.0	140.4	139.3	139.6
Week 15	308.6	301.4	286.9* ↓ 7%	277.1** ↓ 10%
F0 females (gestation period/daily recording)				
Day 3	314.3	305.6	288.6* ↓ 8%	280.7** ↓ 11%
Day 21	422.5	415.1	383.0* ↓ 9%	356.6** ↓ 16%
F1 females (weekly recording)				
Week 0	59.2	54.9	48.6** ↓ 18%	45.4** ↓ 23%
Week 18	307.1	290.5* ↓ 5%	257.8** ↓ 16%	248.1** ↓ 19%
F1 females (gestation period/daily recording)				
Day 3	313.0	297.0	262.7** ↓ 16%	254.1** ↓ 19%
Day 21	426.3	390.6** ↓ 8%	343.9** ↓ 19%	311.4** ↓ 27%

The mean body weights of F0 and F1 parents in the pre-mating dosing periods were significantly reduced at 600 ppm and above. There were also significant reductions in mean body weight during the pre-mating period in F1 parents at 75 ppm. During gestation, the mean body weight of the F0 females was significantly reduced at 600 ppm above and at 75 ppm above in the F1 females. At 75 ppm, the company considered the body weight changes in the females to be small (<10%) or due to the reduced starting weight of the F1 females (body weight gain was similar to the control group). The small reductions in mean body weight in F1 females were not considered toxicologically significant.

There were some reductions in food consumption in the F0 generation at 600 ppm and above during the first 4 weeks of dosing. Significant reductions in food consumption were evident in the F1 generation at 600 ppm and above. At 75 ppm, there were a few small sporadic reductions in food intake in F1 females.

The main haematological and cholinesterase changes in the F0 generation are presented in Table 6.48.

Table 6.48. Mean haematological and cholinesterase determinations in F0 male and female rats

Dose (ppm)	Males				Females			
	0	75	600	1200	0	75	600	1200
RBC (mil/ $\mu$ l)	7.79	8.09	7.92	7.61	7.49	7.50	6.64**	6.36**
Haemoglobin (g/dl)	15.74	15.86	15.68	14.79	15.62	15.43	14.24**	13.73**
Haematocrit (9%)	43.26	44.38	43.56	41.00	43.89	43.68	39.90**	37.73**
RCHO (mu/ml)	1414.50	1230.00 ↓ 13%	1040.77 ↓ 26%	1305.69 ↓ 8%	1537.50	1525.20	1720.00	1636.85
PCHO (mu/ml)	341.2	368.92	300.85 ↓ 12%	295.31 ↓ 13%	879.54	964.20	620.72 ↓ 29%	643.62 ↓ 27%

Key: a) RBC = red blood cells. b) RCHO = red blood cell cholinesterase. c) PCHO = plasma cholinesterase

In F0 parents, the red blood cell count, haemoglobin and haematocrit values were significantly reduced in females at 600 ppm and above. There was a slight reduction in these parameters in males at 1200 ppm. There were no clear compound-related effects on plasma or red blood cell cholinesterase activity.

The main organ weight changes in F0 and F1 parental animals are presented in Table 6.49

Table 6.49. Organ weight changes (absolute and relative to body weight) for the F0 and F1 parents

Dose (ppm)	Males				Females			
	0	75	600	1200	0	75	600	1200
F0 parents								
Spleen: Abs (g)	0.847	0.774	0.793	0.762	0.553	0.567	0.618	0.697**
Rel	0.16	0.14	0.16	0.17	0.17	0.18	0.21**	0.24**
Testes Abs (g)	1.835	1.867	1.812	1.896	-	-	-	-
Rel	0.35	0.33	0.36	0.43**	-	-	-	-
F1 parents								
Spleen: Abs (g)	0.729	0.674	0.684	0.672	0.557	0.578	0.597	0.612
Rel	0.14	0.14	0.16	0.17**	0.17	0.19*	0.22**	0.23**
L/testes Abs (g)	1.890	1.812	1.690	1.690	-	-	-	-
Rel	0.36	0.37	0.39	0.44*	-	-	-	-
R/testes Abs (g)	1.880	1.783	1.797	1.627**	-	-	-	-
Rel	0.36	0.37	0.42*	0.4215*	-	-	-	-
L/kidney Abs (g)	1.792	1.723	1.601*	1.446**	1.080	1.058	0.941*	0.959*
Rel	0.34	0.35	0.37**	0.37**	0.33	0.35	0.34	0.37*
R/kidney Abs (g)	1.832	1.746	1.634	1.419**	1.166	1.073	0.975**	1.007**
Rel	0.35	0.35	0.37	0.37	0.36	0.35	0.35	0.38*

Key: a) Abs= absolute. b) Rel = relative.

The increased mean brain weight values are associated with reduced body weights. Significant increases in mean relative spleen weight were seen in F0 females at 600 ppm and above, in F1 females at 75 ppm and above and F1 males at 1200 ppm. In F0 males, relative mean testes weight was increased in the F0 males at 1200 ppm and in F1 males at 600 ppm and above. In F1 parents, mean relative kidney weight (left) was increased in males at 600 ppm and above and females at 1200 ppm (left and right). No histopathological findings were noted in these organs.

In the F0 females, the increases in relative spleen weight occurred at the same dose levels as the haematological changes but there were no histological changes in the spleen. In the F1 parents, increased relative spleen weight was observed in males and females but the haematological parameters were not evaluated.

There were no effects on the testicular sperm head counts or fertility indices in dose group. The fertility indices and the length of gestation data for the F0 and F1 generations are presented in Table 6.50. The values for the fertility indices are similar but rather low in all dose groups.

Table 6.50. Mean length of gestation and fertility index for the F0 and F1 generations

Dose (ppm)	0	75	600	1200
F0 generation				
Length of gestation (days)	21.8	21.6	21.8	21.9
Fertility index (%)	65.3	57.6	76.9	80.7
F1 generation				
Length of gestation (days)	22.1	21	21.8	21.8
Fertility index (%)	77.5	77.5	79.5	81.6

The litter data for the F1 and F2 pups are presented in Table 6.51 and 6.52.

WARNING: This document forms part of an EC evaluation data package and should not be used in isolation. Registered users must not print or reproduce this document.

Table 6.51 Litter data

Dose (ppm)	0	75	600	1200
F0 generation/F1 pups				
Females pregnant	17/26	15/26	20/26	21/26
Litter size	12.6	13.7	12.5	11.9
Dead pups	0.7	0.3	0.4	0.4
Live pups (day 1)	11.9 <sup>b</sup> (94.8)	13.4 (97.9)	12.1 (96.9)	11.5 (96.1)
Live pups (day 4)	11.8 (99.0)	12.9 (95.9)	11.5 (96.1)	7.8* (67.3*)
<sup>a</sup> Live pups (day 7)	9.7 (100)	9.1 (93.3)	9.5 (98.1)	7.1* (81.2*)
Live pups (day 21)	9.6 (99.4)	9.1 (93.3)	9.2 (95.1)	6.7* (74.3*)
Sex ratio (M:F)	50:50	48:52	53:47	54:46
F1 generation/F2 pups				
Females pregnant	31/40	31/40	31/39	31/38
Litter size	13.2	11.6* <sup>c</sup>	11.0*	11.7*
Dead pups	0.4	0.6	0.3	1.6*
Live pups (day 1)	12.8 (96.9)	<sup>d</sup> 11.0* (96.8)	10.7* (97.9)	10.1* (87.3*)
Live pups (day 4)	12.6 (98.2)	11.0 (96.8)	9.9* (92.8)	7.9* (76.8)
<sup>a</sup> Live pups (day 7)	9.6 (99.4)	9.2 (99.7)	8.9 (99.3)	7.2* (98.4)
Live pups (day 21)	9.6 (98.7)	9.2 (99.0)	8.8 (98.5)	7.1* (97.3)
Sex ratio (M:F)	50:50	48:52	53:47	54:46

Key: a) After culling (pups alive day 7/pups alive day 4 after culling x 100). b) Survival index (%). c) One dam with one dead pup excluded. d) Includes the one dam with one dead pup.

In the F2 pups, significant decreases in the litter size and the number of live pups (day1) were observed at 75 ppm and above. The decrease in litter size and live pups in the 75 ppm dose level was due to one female that delivered only a single dead pup. In addition, a larger F<sub>2</sub> control litter (larger than the F1 generation) contributed to the statistical significance of the 75 ppm F<sub>2</sub> litter size on day 1. Therefore, the decrease in litter size and the number of live pups (day1) were considered to be unrelated to treatment. Pup survival for F1 and F2 pups was reduced at 1200 ppm.

Table 6.52. Mean pup weights (g) at selected time points from birth

Dose (ppm)	0	75	600	1200
F1 pups				
Day 1	6.5 <sup>a</sup> (6.5)	6.2 (6.3)	6.0 (6.0*)	56.6* (5.5*)
Day 4	9.4 (9.3)	8.5* (8.8)	7.9* (7.8*)	6.7* (6.6*)
Day 21	43.2 (43.2)	39.3* (39.6*)	34.2* (34.2*)	30.4* (30.1*)
F2 pups				
Day 1	6.3	6.3	5.7*	5.3*
Day 4	8.9	9.1	7.8*	6.9*
Day 21	40.2	40.2	34.7*	31.7*
Day 49	141.1	138.5	131.2*	117.5*

Key: a) adjusted for litter size on day 1.

During lactation, pup weights in the 600 and 1200 ppm groups were significantly reduced in both generations. In the F<sub>1</sub> generation only, body weights for the 75 ppm group were approximately 9% lower than controls at birth and remained lower by the same proportion throughout the lactation period. This effect became statistically significant by day 4. A comparison of the mean litter size and pup weight is presented in Table 6.53.

Table 6.53. A comparison of the mean litter size and pup weight in the control and low dose groups of both generations

Day	F1 control pups		F1 75 ppm pups	
	Size	Weight (g)	Size	Weight (g)
1	12.6	6.5	13.7	6.2
4	11.8	9.4 (total litter wt = 111)	12.9	8.5* (total litter wt = 110)
7	9.7	14.5	9.1	13.5*
21	9.6	43.2	9.1	39.3*
F2 control pups			F2 75 ppm pups	
1	13.2	6.3	11.6	6.3
4	12.6	8.9	11.0	9.1
7	9.6	14.0	9.2	13.9
21	9.6	40.2	9.2	40.2

The report considered the lower weight of the 75 ppm F<sub>1</sub> pups to be unrelated to treatment for the following reasons: i) There was no effect on body weight at 75 ppm in the F<sub>2</sub> pups at the same concentration whose parents were exposed for a longer period of time. ii) The litter size of the 75 ppm F<sub>1</sub> pups was the largest in either generation (greater nutritional and physiological demands in large litters often lead to reduced pup size and weight). The total litter weight on day 4 (control and 75 ppm groups) is the same but they didn't catch up after the cull. When the body weight of the 75 ppm F<sub>1</sub> pups are compared to those of the F<sub>2</sub> controls (closer in litter size than the F<sub>1</sub> controls), there appears to be no compound effect on mean pup weight. However, these conclusions on pup body weight are only relevant to the combined weights of both sexes; the report summaries did not present the male and female pup body weight data separately.

Table 6.54. The main organ weight changes (absolute and relative to body weight) for the F1 and F2 pups

Dose (ppm)	Males				Females			
	0	75	600	1200	0	75	600	1200
F1 pups								
Spleen:								
Abs (g)	0.401	0.396	0.416	0.539*	0.410	0.361	0.322	0.524
Rel	0.21	0.24	0.35	0.34	0.31	0.24	0.31	0.33
F2 pups								
Spleen:								
Abs (g)	0.465	0.409	0.502	0.601*	0.472	0.409	0.408	0.480
Rel	0.27	0.27	0.30	0.39*	0.31	0.27	0.33	0.37
L/adrenal								
Abs (g)	0.020	0.016*	0.014*	0.014*	0.021	0.025	0.017	0.019
Rel	0.012	0.010	0.009**	0.009*	0.014	0.016	0.013	0.015
R/adrenal								
Abs (g)	0.018	0.015	0.014	0.014	0.019	0.022	0.017	0.017
Rel	0.011	0.010	0.009	0.009	0.013	0.015	0.014	0.013

No test substance-related anatomical changes were noted grossly or microscopically in tissues of parental animals and selected pups in any dose group from either generation.

### Conclusions

Based on decreased body weight at the next highest dose level, a NOAEL of 75 ppm was determined for parental toxicity (equivalent to 4.6 mg/kg bw/day for F0 males, 6.7 mg/kg bw/day for F1 males, 4.8 mg/kg bw/day for F0 females and 6.3 mg/kg bw/day for F1).

A NOAEL of 1200 ppm was determined for reproduction and fertility based on no effects in the F0 and F1 generations (the highest concentration tested).

A NOAEL of 75 ppm was determined for pup growth and development based on the reduced pup weight in the F1 and F2 pups during lactation at 600 ppm and above.

(Lu, 1983)

b)

<b>Report</b>	Busey, D.C. (1968). Three-generation reproduction study Lannate methomyl insecticide. Unpublished DuPont Report No.: MRO 888-19. (in-life phase: not reported).
<b>Test facility</b>	DuPont Haskell Laboratory, P.O. Box 50, Elkton Road, Newark, Delaware, 19714 USA.
<b>Guidelines</b>	Company protocol.
<b>Deviations from guidelines</b>	Not applicable
<b>GLP</b>	No
<b>Test substance</b>	DPX-X1179 Technical.
<b>Batch</b>	X1179-68
<b>Purity</b>	97%
<b>Appearance</b>	White solid
<b>Acceptable</b>	Not acceptable for regulatory purposes on a stand-alone basis (but contains relevant scientific information).

### Material and methods

In a three-generation reproduction study, methomyl was administered to male and female CD rats, starting with approximately one-month-old animals, for approximately 3 months at levels of 0, 0.005 (50 ppm), and 0.01% (100 ppm) in the diet (based on active ingredient). After completion of the feeding period, a reproduction study was conducted with 10 male and 20 female rats within each group in which F1a and F1b litters were produced. Ten male and 20 female weanling rats were selected from the F1b litter in each group and continued on their respective diets for 3 months, at which time they were bred within groups to produce F2a and F2b litters. This same procedure was followed with the F2b litter to produce F3a and F3b litters. Ten male and ten female weanling rats of the F3b litter from the control and each test group were subjected to a histopathological evaluation following gross necropsy.

### Results

There were no test substance-related clinical signs observed during the course of this study. The average weaning weights of the F2 and F3 generation pups fed 0.01% were slightly lower than those of the controls, but the difference was statistically significant only with the F2b litter. A post-weaning growing phase was conducted with female pups from an additional third generation litter. The test litters were fed the test substance for 9 weeks and received only control feed from week 10 through week 14. Body weight gains from week 0 through week 9 for the litter fed 0.01% were significantly lower than those for the control litter; however the decreased growth in the 0.01% test litter was not considered test substance-related. Food consumption through the 14-week growing phase for the 0.01% test litter was significantly lower than control. Body weight gains in the test litter remained lower than the control after the test substance was withdrawn and pups were fed the control diet only.

The indices and litter data obtained for the test groups were comparable with those for the control group. There were no test substance-related effects on litter size or pup survival. No test substance-related gross or histopathologic changes were observed in any of the tissues examined from F3b weanling rats.



## Conclusions

Under the conditions of this study, a NOAEL of 0.01% was determined for parental toxicity and reproduction and fertility (estimated to be equivalent to 100 ppm or 8 mg/kg bw/day). A NOAEL of 0.005% was determined for pup growth and development based on decreased pup weight in F3 pups at of 0.01%, the next highest dose (estimated to be equivalent to 50 ppm or 4 mg/kg bw/day).

(Busey, 1968)

### B.6.6.2 Developmental toxicity studies (AII 5.6.2)

#### a) Rats

<b>Report</b>	Culik, R., Rogers, A.S. (1978). Oral teratogenic study in rats with Lannate (INX-1179). DuPont Report No.: HLR 498-78. (in-life phase: 1978).
<b>Test facility</b>	DuPont Haskell Laboratory, P.O. Box 50, Elkton Road, Newark, Delaware, 19714 USA.
<b>Guidelines</b>	Directive 87/302/EEC Part B: Teratogenicity.
<b>Deviations from guidelines</b>	An acclimation period at the test facility was not conducted since the rats were inseminated by the supplier. The test substance was administered in the diet instead of by gavage. The sex of the foetuses was not reported.
<b>GLP</b>	No
<b>Test substance</b>	DPX-X1179 Technical.
<b>Batch</b>	X1179-255
<b>Purity</b>	99%
<b>Appearance</b>	White solid
<b>Acceptable</b>	Yes

## Material and methods

Pregnant Chr-CD female rats (25/dose group) were administered diet containing methomyl at concentrations of 0, 50, 100 or 400 ppm on gestation days 6-15. This was equivalent to 0, 4.9, 9.4, and 33.9 mg/kg bw/day, respectively. The animals were sacrificed on gestation day 21.

Parameters evaluated in dams were body weight, food consumption, survival, clinical signs, reproductive outcomes (including corpora lutea in each ovary, implantation sites in each horn, number and location of foetuses) and gross pathology. Parameters evaluated in foetuses were body weight, incidences of dead foetuses and/or foetal resorptions, crown-rump length of each live foetus, and incidences of external, visceral, and skeletal malformations and variations.

## Results

No compound-related clinical signs of toxicity or mortality were observed in any of the test groups during the study. The body weight and food consumption values are presented in Table 6.55.

Table 6.55. Mean body weights (g) and food consumption (g/rat/day) during gestation

Dose (ppm)	0	50	100	400
Initial body weight	176.7	171.4	173.4	171.7
Gestation day 6	205 (17.9)	201 (18.1)	200 (17.7)	200 (18.0)
Gestation day 16	283 (24.0)	279 (23.5)	277 (22.5)	261 (↓ 8%) (19.6)
Gestation day 21	361 (30.0)	357 (29.3)	357 (29.4)	343 (↓ 5%) (28.2)

In the top dose group, the number of pregnant females was noticeably lower than in the control group (Table 6.56) but this appears to be a pre-exposure phenomenon. The number of corpora lutea/pregnant female, implantations/litter, live foetuses/litter, partial and total resorptions, foetal crown-rump length and foetal weight (both sexes combined) were not different from those of the concurrent controls. The male and female foetal weights were not evaluated separately and the sex ratio was not determined.

Table 6.56. Anomalies and malformations in pups

Dose (ppm)	0	50	100	400
Gross findings				
Number of litters	23	21	25	19
Number of pups	195	174	218	179
Mean litter size	8.5	8.3	8.7	9.3
Runts	0	1	1	1
Subcutaneous hematomas in pups (litters)	7 (6)	13 (4)	17 (13)	5 (4)
Petechial haemorrhages in pups (litters)s	7 (6)	4 (4)	10 (8)	19 (9)
Visceral findings				
Number of litters (foetuses)	23 (93)	20 (84)	25 (102)	19 (86)
Apparent hydronephrosis in pups (litters)	3 (3)	10 (6)	6 (4)	2 (2)
Peliosis of liver in pups (litters)	2 (1)	5 (4)	5 (3)	0
<sup>b</sup> Exencephalocele in pups (litters)	0	0	1 (1)	0
<sup>c</sup> Hydrocephalous in pups (litters)	0	0	1 (1)	0
Skeletal findings				
Number of litters (foetuses)	22 (102)	21 (90)	25 (116)	19 (93)
14 <sup>th</sup> rib(s) in pups (litters)	2 (1)	3 (3)	2 (2)	6 (5)

Key: a) Gestation day. b) Also includes bilateral hydronephrosis and misshapen heart. c) Small foetus (2.5 g) with fused aorta and pulmonary artery.

One runt was found in each of the three test groups. Small subcutaneous haematomas and petechial haemorrhages on various parts of the body were found in foetuses from litters of all groups. Most anomalies and malformations found in the soft-tissue sectioning were isolated occurrences with two exceptions: apparent hydronephrosis (found in all groups at approximately the same rate) and peliosis (observed in all but 400 ppm foetuses at a rate similar to controls) of the liver. There was a small increase

in the number of litters with 14<sup>th</sup> rib(s) at 400 ppm, a dose that produced a 20% reduction in maternal body weight.

No compound-related gross morphological changes were observed in the dams or in the pups at sacrifice.

### Conclusion

A NOEL of 100 ppm (9.4 mg/kg bw/day) was determined for maternal toxicity based on reduced body weight at 400 ppm. A NOAEL of 400 ppm (33.9 mg/kg bw/day) was determined for direct foetal effects, the highest dose tested.

(Culik and Rogers, 1978)

#### b) Rabbits

<b>Report</b>	Feussner, E.L., Hoberman, A.M., Christian, M.S. (1983). Embryo-fetal toxicity and teratogenicity study of methomyl in the rabbit. Unpublished DuPont Report No.: HLO 331-83. (in-life phase: March 1983 to April 1983).
<b>Test facility</b>	Argus Research Laboratories, Inc., 935 Horsham Road, Horsham, Pennsylvania, 19044 USA
<b>Guidelines</b>	Directive 87/302/EEC Part B: Teratogenicity.
<b>Deviations from guidelines</b>	Dosing was conducted on gestation days 7-19 instead of days 6-18.
<b>GLP</b>	No
<b>Test substance</b>	DPX-X1179 Technical
<b>Batch</b>	X1179-255
<b>Purity</b>	98.7%
<b>Appearance</b>	White solid
<b>Acceptable</b>	Yes

### Material and methods

Artificially inseminated (four bucks) DLI:NZW female rabbits (20/dose group) were administered oral (gavage) doses of 0, 2, 6 or 16 mg/kg bw/day in deionised water on gestation days 7 -19. The dose volume was 5 ml/kg bw. Dose selection was based on a preliminary study using 8, 16 and 24 mg/kg bw/day. All surviving dams were sacrificed on gestation day 29.

Parameters evaluated in dams were body weight, body weight gain, food consumption, survival, clinical signs, reproductive outcomes (including corpora lutea, placement of implantations, early and late resorptions, live and dead fetuses, gravid and absolute uterine weights), and gross pathology. Parameters evaluated in fetuses were body weight, incidences of dead fetuses and/or foetal resorptions, and incidences of external, visceral and skeletal malformations and variations.

## Results

Nine females died during the study (Table 6.57), 7 compound-related deaths and one incidental death at the top dose. There was one death in the mid dose group, possibly caused by gavage dosing but there was evidence of excessive salivation in the decedent. This death occurred 8 days following the cessation of dosing so is unlikely to be related to methomyl administration.

Table 6.57. Mortality data

Dose (mg/kg bw/day)	0	2	6	16
Deaths	0	0	<sup>a</sup> 1 (day 27)	7 (days 7, 8, 9, 11 17, 18 and 19) plus 1 on day 2 <sup>b</sup> .
Aborted	-	1	-	-
Delivered naturally	-	-	1	-

Key: a) An opening in the stomach wall near to the cardiac sphincter was revealed at necropsy but there was evidence of excessive salivation. b) On animal died before administration of the test substance and was presumed not pregnant for the calculations.

At 16 mg/kg bw/day, the clinical signs of toxicity included tremors, hyperactivity, body jerks, excess salivation (discharge around the mouth and/or nose at necropsy of decedents), aggressive behaviour, hyperpnea, convulsions, ataxia, impaired or lost righting reflex, skin lesions, dyspnea, hypersensitivity, pin-point pupils and vocalisation. The incidence of test substance-related anorexia was significantly decreased for rabbits fed 16 mg/kg bw/day. This observation was considered to be a rebound in food consumption after the test substance was no longer administered.

A small compound-related increase in maternal body weight gain occurred between days 0 and 29 of gestation; however only the gain of the 16 mg/kg bw/day dose group differed significantly from controls. This difference was due to the decreased incidence of anorexia of the dams in the 16 mg/kg bw/day group during the latter portion of gestation.

Table 6.58. Reproductive and foetal data

Dose (mg/kg bw/day)	0	2	6	16
No. pregnant/No. inseminated	16/20	16/20	17/20	16/20
No. deaths	0/20	0/20	1/0	8/20
No. aborted	-	1/16	-	-
No. delivered naturally	-	-	1/17	-
No. total resorptions	2/16	-	-	-
No. litters	14/16	15/16	15/17	9/16
Mean No of resorptions	0.7 (0.6) <sup>c</sup>	0.8 (1.4)	0.5 (0.6)	0.7 (0.9)
<sup>b</sup> Mean No of corpora lutea	10.3 (3.7)	10.8 (2.7)	11.2 (3.1)	9.7 (2.1)
<sup>b</sup> Mean No of implants	8.6 (3.4)	8.1 (3.3)	7.8 (2.4)	8.2 (2.2)
<sup>a</sup> Total No. foetuses (live + dead)	127	110	109	68
<sup>a</sup> No. live foetuses	127	110	108	68
<sup>a</sup> No. of dead foetuses	-	-	1	-
<sup>d</sup> Mean No of live foetuses /litter	9.1	7.3 [↓ 19.8%]	7.3 [↓ 19.8%]	7.6 [↓ 16.5%]
<sup>a</sup> Mean weight (g)	38.8 (6.3)	44.1* (7.0)	43.3* (8.4)	46.0** (4.2)

Key: a) Excludes rabbits that died, aborted, delivered, were not pregnant or had total resorptions. b) Excludes rabbits that died, aborted, delivered, were not pregnant; included those with total resorption. c) Standard deviation. d) 7.9 = 127/16 (denominator includes two dams with total resorptions) whereas 9.1 = 127/14 (denominator excludes two dams with total resorptions).

Reproductive outcomes: A test substance-related increase in average foetal body weight occurred; the difference from the control value was statistically significant only for the 6 and 16 mg/kg bw/day dose groups. This increase in foetal body weight was not considered to be an adverse effect since it was not observed to be due to the presence of oedema. There was no effect on the foetal sex ratio.

The mean number of live foetuses per dam is noticeably reduced at 2.0 mg/kg bw/day and above. However, is probably due to the high value obtained for the control animals. The notifier has provided historical control data for the contract laboratory and their own laboratory. A mean of 7.6 live foetuses/litter (range 5.6-11.5) was obtained for 10 studies conducted by the contract laboratory in the 1985. For the period of 1983-1995, the mean number of live foetuses/litter was 6.0 with a range of 4.0-9.0 for the company's laboratory. Based on the historical data, the absence of a dose-response relationship and no increases in resorptions or dead foetuses, the test material did not affect litter size in this study.

Malformations (external, visceral and skeletal) were observed in a total of 6/5, 5/5, 7/5 and 6/4 (foetuses/litter) at 0, 2, 6 and 16 mg/kg bw/day, respectively, but there as no indications of a treatment related effect. The average percent of malformed foetuses per litter was  $4.4 \pm 6.61$ ,  $3.8 \pm 5.85$ ,  $9.5 \pm 18.19$  and  $5.9 \pm 10.06$  at the same dose levels. However, it is possible that the low number of pregnant females at the top dose might mask a dose response relationship.

No gross external, soft tissue or skeletal malformations, developmental variations or variations due to retarded development observed for foetuses were attributed to test substance administration.

A NOAEL of 6 mg/kg bw/day was determined for maternal effects based on deaths, decreased body weight and clinical signs of cholinesterase activity at 16 mg/kg bw/day, the next highest dose. The NOAEL for foetal effects was 16 mg/kg bw/day, the highest dose tested.

(Feussner, Hoberman and Christian, 1983)

### **B.6.6.3 Summary of reproductive toxicity**

The NOEL/NOAELs and LOELs are summarised in Table 6.81. The purity of the methomyl tested ranged between 97-99% for the early three generation study (1960s) and developmental studies but it was not specified for the more recent two generation study.

In the two-generation reproduction study, the NOAEL for parental toxicity was 75 ppm based on reduced body weight at the next highest dose. There were no effects on reproduction or fertility. A NOAEL of 75 ppm was determined for pup growth and development based on the reduced pup weight in the F1 and F2 pups during lactation at 600 ppm and above. In the three generation reproduction toxicity study, an estimated NOAEL of 100 ppm was determined for parental toxicity and reproduction and fertility (estimated to be equivalent to 8 mg/kg bw/day). An estimated NOAEL of 50 ppm (equivalent to 4 mg/kg bw/day) was determined for pup growth and development based on decreased pup weight in F3 pups at 100 ppm, the next highest dose.

In the rat developmental toxicity study, the NOAEL for maternal effects was 9.4 mg/kg bw/day based on body weight effects, and decreased food consumption at 33.9 mg/kg bw/day. The NOAEL for fetuses was 33.9 mg/kg bw/day, the highest dose tested. In the rabbit developmental study, a maternal NOAEL of 6 mg/kg bw/day was determined based on deaths, decreased body weight and clinical signs of cholinesterase activity at 16 mg/kg bw/day. The NOAEL for foetal effects was 16 mg/kg bw/day, the highest dose tested.

There was no evidence of methomyl-induced teratogenic activity in rats or rabbits.

### **B.6.7 Neurotoxicity studies (AII 5.7)**

#### **B.6.7.1 Delayed neurotoxicity studies**

The majority of the literature indicates that carbamates do not bind to neuropathy target esterase (NTE) (Casarett and Doull's Toxicology, 1996 The Basic Science of Poisons, Fifth Edition, Ed. C. Klaassen, McGraw-Hill, New York) and do not cause delayed neurotoxicity. However, a test for delayed neurotoxicity in hens was conducted some years ago. Although this study does not meet the current testing guidelines, it does provide scientific evidence that methomyl does not cause delayed neurotoxicity at dose levels up to 200 mg/kg bw.

<b>Report</b>	Krauss, W.C, Stula, E.F. (1967). Oral LD <sub>50</sub> and delayed paralysis test (hens). Unpublished DuPont Report No.: HLR 161-67. (in-life phase: 1967).
<b>Test facility</b>	DuPont Haskell Laboratory, P.O. Box 50, Elkton Road, Newark, Delaware, 19714 USA
<b>Guidelines</b>	Company protocol
<b>Deviations from guidelines</b>	Not applicable
<b>GLP</b>	No
<b>Test substance</b>	DPX-X1179 Technical.
<b>Batch</b>	X1179-68
<b>Purity</b>	97%
<b>Appearance</b>	White solid
<b>Acceptable</b>	Not acceptable for regulatory purposes on a stand-alone basis (but contains relevant scientific information).

### Materials and methods

Methomyl (5% suspension in acetone/water 1:10) was administered as an oral dose to 1 or 5 hens per group at dose levels shown in Table 6.60. The acute oral LD<sub>50</sub> value (without atropine pre-treatment) was calculated to be 28 mg/kg bw. Doses higher than the LD<sub>50</sub> were administered by pre-treating the hens with 10 mg/kg bw atropine. Triorthocresyl phosphate was administered as a positive control at a dose of 500 mg/kg bw (10 hens).

### Results

At 20-40 mg/kg bw/day, salivation and respiratory irregularities occurred and the hens died within 10 minutes of dosing. At doses of 60-200 mg/kg bw methomyl, following pre-treatment with atropine, mortality did not occur; however, convulsions and reduced egg production were observed. The hen administered 200 mg/kg bw did not eat for 3 days.

Table 6.59. Mortality and delayed toxicity data

Dose methomyl (mg/kg bw)	No. Hens	Dose Atropine	Mortality	Paralysis
20	5	-	1/5	NAD
40	5	-	4/5	NAD
60	1	10	0	NAD
90	1	10	0	NAD
120	1	10	0	NAD
200	1	10	0	NAD
500 mg/kg bw triortho-cresyl phosphate	10	-	0/10	10/10

Key: a) NAD = No abnormalities detected.

There was no evidence of wing or leg paralysis during the 21-22 day recovery period, and no macroscopic or microscopic changes in the sciatic nerve. Appropriate results were obtained with the positive control material (leg paralysis and axonal degeneration of sciatic nerve).

## Conclusions

Under the conditions of this study (summary only), methomyl did not cause delayed neurotoxicity in hens at dose levels above the unprotected LD50 value.

(Krauss and Stula, 1967)

### B.6.7.2 Acute and repeat dose neurotoxicity studies

a)

<b>Report</b>	Mikles, K.A. (1998a). Methomyl technical (DPX-X1179-512): Acute oral neurotoxicity study in rats. Unpublished DuPont Report No.: HL-1998-01080 (2 volumes). (in-life phase: September 1997).
<b>Test facility</b>	DuPont Haskell Laboratory, P.O. Box 50, Elkton Road, Newark, Delaware, 19714 USA
<b>Guidelines</b>	USEPA 81-8 (1982).
<b>Deviations from guidelines</b>	Not applicable
<b>GLP</b>	Yes
<b>Test substance</b>	DPX-X1179 Technical.
<b>Batch</b>	X1179-512
<b>Purity</b>	98.6%
<b>Appearance</b>	White solid
<b>Acceptable</b>	Yes

## Materials and methods

Groups of Crl:CD BR rats (52 rats/sex/group) were administered a single oral (gavage) dose of methomyl at 0, 0.25, 0.5, 0.75, and 2 mg/kg bw in commercially supplied bottled water. The volume of administration was 10 ml/kg. Dose selection was based on a reversibility study and a pilot study. In the reversibility study, clinical signs (40/sex/dose) and cholinesterase activity (10/sex/dose) were evaluated at 0.5, 2, 3 and 4 hours after dosing with 3 mg/kg bw. Peak effects were observed at 30 minutes post-dose and included tremors in males and females, lacrimation in one male and cholinesterase inhibition. Plasma, erythrocyte and brain cholinesterase activities were inhibited 27, 56 and 46% in male rats and 10, 41 and 39% in female rats, respectively. In the pilot study, a modified functional observation battery (FOB) and motor activity assessments were carried out (5/sex/dose) at 1 mg/kg bw. At 30 minutes, tremors, low posture, abnormal gait and uncoordinated righting reflex were observed.

The experimental design of the main study is presented in Table 6.60.



Table 6.60. Experimental design (the number of rats in each group that underwent specific evaluations)

Assessment	Neurobehaviour	Neuropathology	<sup>a</sup> Blood	<sup>b</sup> Brain
Baseline	12	0	10	0
Test day 1	<sup>c</sup> 12	0	<sup>c</sup> 10	<sup>c</sup> 10
Test day 2	0	0	10	10
Test day 8	12	0	0	0
Test day 15	12	0	0	0
Test day 16	0	6	0	0

Key: a) Evaluation of plasma and red blood cell cholinesterase activity. b) Evaluation of cholinesterase activity in the whole brain. c) Assessment was conducted at approximately 30 minutes post-dosing (the time of peak effect).

A neurobehavioral test battery, consisting of motor activity and functional observational assessments (FOBs), was conducted on 12 rats/sex/group prior to dosing, 30 minutes after dosing (day 1), and on days 8 and 15.

Forty rats/group were designated as the clinical pathology subgroup. Cholinesterase activity in plasma and erythrocytes was measured for the first ten clinical pathology rats/group prior to test substance administration to establish baseline levels. Cholinesterase activity in brain tissue, plasma, and erythrocytes was measured again in the same ten animals/group at approximately 30 minutes post-dosing on test day 1. Cholinesterase activity was measured in the second ten clinical pathology rats/group on test day 2. The last twenty clinical pathology rats/group were dosed and evaluated for clinical signs on test days 1 and 2; however, they were not needed for cholinesterase assessments on test days 8 and 15 and consequently removed from the study. The blood samples were stored on ice until analysed. The brain tissue was stored on ice until frozen at -70°C and analysed at a later date. All cholinesterase determinations were carried out using a Hitachi 717 analyser and Boehringer Mannheim reagents (based on the Ellman method).

Other parameters evaluated included body weight, body weight gain, food consumption, food efficiency, clinical signs, and microscopic evaluation of the central and peripheral nervous system and selected muscle tissues (control and top dose groups only). Tissues for routine and high resolution light microscopy were fixed by whole-body *in situ* perfusion.

## Results

Analysis confirmed that the concentration and stability of the test substance in the dosing solutions was acceptable.

No compound-induced mortality occurred during this study. Clinical signs of toxicity observed in the male and female animals of the clinical pathology subgroup administered 2 mg/kg bw of the test substance included tremors, salivation and/or lacrimation approximately 30 minutes post dosing on test day 1. Chromodacryorrhea was observed on test day 2 in 2 mg/kg bw rats but was considered to be secondary to stress. One female exhibited tremors at 0.75 mg/kg bw.

Table 6.61. Summary of the clinical observations

Dose	0	0.25	0.5	0.75	2
<b>Males</b>					
<sup>a</sup> Discharge eye	0	0	0	0	2/12 (3)*
<sup>b</sup> Tremors	0	0	0	0	5/40 (1)*
<b>Females</b>					
<sup>b</sup> Discharge eye	0	0	0	0	1/40 (1)
<sup>b</sup> Tremors	0	0	0	1/40 (1)	5/40 (1)*
<sup>b</sup> Salivation	0	0	0	0	1/40 (1)

Key: a) Neurotoxicity sub-study. b) Clinical chemistry sub-study. c) The numbers in parentheses indicate the median for days-on-test when the sign was first observed.

Body weights and food consumption of male and female neurotoxicity subgroup rats were unaffected by the test substance. Mean body weight gains of male rats were also unaffected. Body weight gains were decreased in female rats administered 2 mg/kg bw during the interval of test days 2-8 but had returned to control values during the interval of test days 8-15.

The functional observational battery on test day 1 revealed tremors and lacrimation in males administered 2 mg/kg bw. No test substance-related effects were observed on test days 8 and 15. There were no test substance-related effects on forelimb grip strength, hindlimb grip strength, and hindlimb foot splay. Motor activity scores of treated rats were unaffected by the test substance.

The plasma, erythrocyte and brain cholinesterase activities are presented in Table 6.62. Inhibition of plasma, RBC, and brain cholinesterase activity was detected in males and females administered 0.5, 0.75, and 2 mg/kg bw. The inhibition was observed on test day 1 approximately 30 minutes after dose administration. All cholinesterase activity levels were comparable with the control values on test day 2.

Table 6.62. Mean cholinesterase activities (approximately 30 minutes post dosing)

Dose (mg/kg bw)	Day 0	Day 1	Day 2	Day 0	Day 1	Day 2
	Males			Females		
Plasma cholinesterase (U/l)						
0	456	456	450	934	840	699
0.25	425	380 (↓ 17%)	454	886	749 (↓ 11%)	860
0.50	460	397 (↓ 13%)	441	829	649 (↓ 23%)	769
0.75	456	349 (↓ 23%)*	433	817	562 (↓ 33%)*	854
2.00	435	263 (↓ 42%)*	442	957	602 (↓ 28%)*	955
Erythrocyte cholinesterase (U/l)						
0	2026	1716	1928	2046	2426	2146
0.25	2066	1746	1912	1986	2074 (↓ 15%)	2046
0.50	2062	1634 <sup>a</sup> (↓ 5%)	1848	2036	1826 (↓ 25%)*	1994
0.75	1904	1206 (↓ 30%)	2240	2250	1492 (↓ 38%)*	2024
2.00	2294	928 (↓ 46%)*	2214	2096	1054 (↓ 57%)*	1988
Brain cholinesterase (U/g)						
0	-	11.68	12.09	-	12.44	12.17
0.25	-	11.00	11.80	-	11.99	12.19
0.5	-	9.47 (↓ 19%)*	11.93	-	9.93 (↓ 20%)*	11.81
0.75	-	8.71 (↓ 25%)*	12.33	-	8.73 (↓ 30%)**	11.98
2.0	-	6.24 (↓ 47%)*	12.20	-	6.08 (↓ 51%)**	12.03

Key: a) Percent decrease from control value.

The microscopic examination revealed no adverse findings in the tissues evaluated.

## Conclusions

A NOAEL of 0.25 mg/kg bw was determined based on reversible dose-related inhibition of erythrocyte and brain cholinesterase (>20%) at dose levels of 0.5 mg/kg.

(Mikles, 1998a)

b)

<b>Report</b>	Mikles, K.A. (1998b). Methomyl technical (DPX-X1179-512): subchronic oral neurotoxicity study in rats. Unpublished DuPont Report No.: HL-1998-01639 (2 volumes). (in-life phase: January 1998 to may 1998).
<b>Test facility</b>	DuPont Haskell Laboratory, P.O. Box 50, Elkton Road, Newark, Delaware, 19714 USA
<b>Guidelines</b>	USEPA 81-7 (1982).
<b>Deviations from guidelines</b>	Not applicable
<b>GLP</b>	Yes
<b>Test substance</b>	DPX-X1179 Technical.
<b>Batch</b>	X1179-512
<b>Purity</b>	98.6%
<b>Appearance</b>	White solid
<b>Acceptable</b>	Yes

### Materials and Methods

Groups of Crl:CD BR rats (42 rats/sex/group) were administered diet containing methomyl at concentrations of 0, 20, 50, 150 or 1500 ppm for 90-days. Dose selection was based on previous toxicity studies.

The experimental design of the study is presented in Table 6.63.

Table 6.63. Experimental design (the number of rats in each group that underwent specific evaluations)

Assessment	Neurobehaviour	Neuropathology	<sup>a</sup> Blood	<sup>b</sup> Brain
Baseline	12	0	10	0
Week 4	12	0	10	10
Week 8	12	0	10	10
Week 13	12	0	10	10
Week 14	0	6	0	0

Key: a) Evaluation of plasma and red blood cell cholinesterase activity. b) Evaluation of cholinesterase activity in the whole brain. c) Assessment was conducted at approximately 30 minutes post-dosing (the time of peak effect).

Parameters evaluated included body weight, body weight gain, food consumption, food efficiency and clinical signs. Cholinesterase activities in plasma and red blood cells were measured for the first 10 clinical pathology rats/sex/group prior to test substance-administration to establish baseline values. Plasma, red blood cell, and brain cholinesterase activity was measured in 10 rats/sex/group in weeks 4, 8, and 13. The blood samples were stored on ice until analysed. The brain tissue was stored on ice until frozen at -70°C and analysed at a later date. All cholinesterase determinations were carried out using a Hitachi 717 analyser and Boehringer Mannheim reagents (based on the Ellman method).

A neurobehavioral test battery consisting of motor activity and functional observational assessments was conducted one week prior to the first week of dietary administration and during weeks 4, 8, and 13 on 12 rats/sex/group. After the final neurobehavioral assessments, the rats were perfused *in situ* with fixative. The

peripheral and central nervous system and selected muscle tissues from control and high dose rats (6 rats/dose) were examined microscopically.

## Results

Analysis confirmed the concentration, stability and homogeneity of the test substance in the diet were acceptable. The daily intakes are presented in Table 6.64.

Table 6.64. Mean daily intakes of test substance

Dose (ppm)	Males (mg/kg bw/day)	Females (mg/kg bw/day)
0	0	0
20	1.29	1.48
50	3.14	3.85
150	9.42	11.20
1500	94.90	113.00

No test compound-related mortality occurred during the study. Clinical signs of toxicity observed in the 1500 ppm dose group included tremors in males and females, increased aggressive behaviour and hyperreactivity in males and increased alopecia in females.

Table 6.65. Body weight and food consumptions data (0-91 days)

Dose (ppm)	0	20	50	150	1500
Males					
Bodyweight gain (g)	339.9	332.1	317.2	329.7	218.0*
Food consumption (g/day)	25.2	25.2	25.4	25.1	19.7*
Food efficiency	0.148	0.144	0.138	0.144	0.122*
Females					
Bodyweight gain (g)	145.9	134.2	129.1	130.4	89.2*
Food consumption (g/day)	18.3	18.3	17.9	17.8	15.4*
Food efficiency	0.087	0.080	0.079	0.080	0.064*

Body weights were 24% and 19% lower than controls in the 1500 ppm males and females respectively. Body weight gains were significantly lower than controls in the 1500 ppm males and females. Statistically significant and test substance-related decreases in food consumption and food efficiency occurred in the 1500 ppm males and females.

Changes in the functional observational battery parameters are presented in Tables 6.66, 6.67 and 6.68. Reductions in forelimb and hindlimb grip strength were seen in 1500 ppm males at 13 weeks only. The forelimb and hindlimb grip strength scores of females were unaffected by treatment at all dose levels.

Table 6.66. Grip strength in males (mean of three trials)

Dose (ppm)	0 (n=12)	20 (n=12)	50 (n=12) <sup>a</sup>	150 (n=12)	1500 (n=12)
Male forelimb grip strength (kg)					
Baseline	0.32 (0.10) <sup>b</sup>	0.30 (0.10)	0.32 (0.11)	0.36 (0.08)	0.34 (0.09)
Week 4	0.65 (0.16)	0.64 (0.25)	0.57 (0.15)	0.54 (0.10)	0.51 (0.13)
Week 8	0.85 (0.16)	0.92 (0.21)	0.91 (0.18)	0.87 (0.22)	0.73 (0.22)
Week 13	0.96 (0.21)	0.87 (0.28)	0.96 (0.20)	0.80 (0.11)	0.75 (0.19)*
Male hindlimb grip strength (kg)					
Baseline	0.27 (0.08)	0.27 (0.06)	0.27 (0.06)	0.28 (0.07)	0.29 (0.09)
Week 4	0.65 (0.15)	0.67 (0.19)	0.77 (0.19)	0.73 (0.19)	0.58 (0.17)
Week 8	0.92 (0.14)	0.90 (0.19)	0.97 (0.24)	0.91 (0.16)	0.82 (0.22)
Week 13	0.94 (0.20)	0.80 (0.19)	0.82 (0.22)	0.80 (0.18)	0.67 (0.18)*

Key: a) n = 11 on weeks 8 and 13. b) Standard deviation.

Table 6.67. Summary of the changes in the FOB parameters in male rats

Dose (ppm)	0 (n=12)	20 (n=12)	50 (n=12) <sup>a</sup>	150 (n=12)	1500 (n=12)
Outside home cage-difficult removal					
Baseline	0	0	0	0	0
Week 4	0	0	0	0	3*
Week 8	0	0	0	0	1
Week 13	0	0	0	0	1
Outside home cage-difficult handling					
Baseline	0	0	0	0	0
Week 4	0	0	0	1	4*
Week 8	0	0	0	0	3*
Week 13	0	0	0	0	1
Outside home cage-vocalisation					
Baseline	0	0	0	0	0
Week 4	0	0	0	1	1
Week 8	0	0	2	0	2
Week 13	0	0	0	0	1
Outside home cage-ptosis					
Baseline	0	0	0	0	0
Week 4	0	0	1	0	1
Week 8	0	0	0	0	3*
Week 13	0	0	0	0	0
Open field-abnormal gait					
Baseline	0	0	0	0	0
Week 4	0	0	0	0	0
Week 8	0	0	1	0	1
Week 13	0	0	0	0	0
Motor activity monitor-absent papillary response					
Baseline	0	0	0	0	0
Week 4	0	0	0	0	5*
Week 8	0	0	0	0	6*
Week 13	0	0	0	0	5*

Key: a) n = 11 on weeks 8 and 13. b) Standard deviation.

At 1500 ppm, the findings common to both sexes included difficulty removing the rats from their cages, difficulty handling the rats, ptosis and abnormal papillary responses. Low arousal and abnormal gait were observed in females at 1500 ppm. The statistically significant urination and defecation effects in females are not considered to be treatment-related. Carbamates are known to increase rather than decrease urination. The decreases in defecation might be a result of lower food consumption.

Table 6.68. Summary of the changes in the FOB parameters in female rats

Dose (ppm)	0 (n=12)	20 (n=12)	50 (n=12) <sup>a</sup>	150 (n=12)	1500 (n=12)
Outside home cage-difficult removal					
Baseline	1	0	0	0	0
Week 4	0	0	0	0	1
Week 8	0	0	0	0	2*
Week 13	0	0	0	0	0
Outside home cage-difficult handling					
Baseline	0	0	0	0	0
Week 4	0	0	0	0	1
Week 8	0	0	0	1	2*
Week 13	0	0	0	0	0
Outside home cage-vocalisation					
Baseline	0	0	0	1	0
Week 4	0	0	0	0	2*
Week 8	1	0	0	1	2
Week 13	0	0	0	0	0
Outside home cage-ptosis					
Baseline	0	0	0	0	0
Week 4	0	0	0	1	2*
Week 8	0	0	0	0	2*
Week 13	0	0	0	0	0
Open field-abnormal gait					
Baseline	0	0	0	0	0
Week 4	0	1	0	0	1
Week 8	0	2	0	1	4*
Week 13	0	0	0	0	0
Open field-low arousal					
Baseline	0	0	0	0	0
Week 4	0	0	1	1	3*
Week 8	3	1	3	2	2
Week 13	1	1	1	0	0
Open field-urination					
Baseline	2	2	4	5	2
Week 4	0	0	0	2	3*
Week 8	2	0	1	0	0
Week 13	3	0	2	0	2
Motor activity monitor-absent papillary response					
Baseline	0	0	0	0	0
Week 4	1	1	0	0	4*
Week 8	0	0	0	0	2*
Week 13	0	0	0	0	4*
Motor activity monitor-urination					
Baseline	4	3	6	4	6
Week 4	9	7	6	4*	5*
Week 8	6	4	1	3	1*
Week 13	5	1	1	5	3
Motor activity monitor-defecation					
Baseline	1	3	4	5	4
Week 4	4	3	1	1	1*
Week 8	4	4	2	2	0*
Week 13	5	3	2	3	0*

Key: a) n = 11 on weeks 8 and 13. b) Standard deviation. c) \* statistically significant by Cochran-Armitage trend test (p<0.05).

The results of the cholinesterase determinations are presented in Table 6.69. A minimal to mild decrease in brain cholinesterase activity was seen in both sexes at 1500 ppm.

Table 6.69. Summary of the cholinesterase determinations in rats

Dose (ppm)	0 (n=12)	20 (n=12)	50 (n=12) <sup>a</sup>	150 (n=12)	1500 (n=12)
Male plasma cholinesterase (U/l)					
Pre-test	448.8	493.8	486.3	484.6	478.2
Week 4	364.0	414.6	399.6	410.2	406.6
Week 8	384.7	428.2	395.7	397.0	353.9
Week 13	425.7	399.7	427.3	413.2	440.1
Male erythrocyte cholinesterase (U/l)					
Pre-test	2084	2172	2036	2302	1934
Week 4	1790	1838	1360	1734	1992
Week 8	1497	1478	1610	1872	1652
Week 13	1514	1688	1684	1520	2118
Male brain cholinesterase (U/g)					
Pre-test	Nm	Nm	Nm	Nm	Nm
Week 4	11.29	11.29	11.28	11.35	10.41
Week 8	11.24	10.90	10.98	10.82	9.14 (↓ 17%)
Week 13	10.65	10.37	10.53	10.81	10.09
Female plasma cholinesterase (U/l)					
Pre-test	551.1	584.8	554.7	519.2	592.7
Week 4	1379.5	1476.2	1404.6	1263.8 (↓ 8%)	1273.8 (↓ 8%)
Week 8	1697.4	2059.8	2205.6	1908.0	1504.3
Week 13	2009.7	1869.2	2305.3	1967.9	1796.5
Female erythrocyte cholinesterase (U/l)					
Pre-test	2090	2046	1706	1892	1906
Week 4	1834	1860	1568	1780	1974
Week 8	1718	1546	1728	1762	2336
Week 13	2228	2006	1756	1784	2332
Female brain cholinesterase (U/g)					
Pre-test	Nm	Nm	Nm	Nm	Nm
Week 4	11.95	11.49	11.65	11.24	10.79 (↓ 10%)
Week 8	11.57	11.18	11.36	11.61	11.15
Week 13	11.15	10.72	10.86	10.71	10.80

Key: a) Nm = not measured. b) ↓ percent decrease compared to control value.

There were no test substance-related microscopic findings in the tissues examined.

## Conclusions

Based on reduced body weights, food consumption and food efficiency, decreased rip strength and clinical signs at 1500 ppm, a NOAEL of 150 ppm was determined for both sexes (equivalent to 9.42 and 11.2 mg/kg bw/day, respectively).

(Mikles, 1998b)



### B.6.7.3 Summary of the neurotoxicity studies

The NOEL/NOAELs and LOELs are summarised in Table 6.81.

There was no evidence of delayed toxicity in the hen test.

Following acute oral (gavage) dosing with methomyl (purity 98.6%) in water, clinical signs of systemic toxicity and cholinesterase inhibition (plasma, erythrocyte and brain activities) were observed in both sexes at peak exposure (30 minutes post dosing). There were no effects on body weight, food consumption, the functional observation battery parameters or motor activity. It was also noted that fore and hind limb grip strength was not affected by treatment. A NOAEL of 0.25 mg/kg bw was determined for reversible dose-related brain cholinesterase activity at the next highest dose. Clinical signs were evident at 1 mg/kg bw in a pilot study (tremors, low posture, abnormal gait and uncoordinated righting reflex).

Following dietary administration for 90-days (methomyl purity 98.6%), there were effects on body weight, food consumption, clinical signs of systemic toxicity, brain cholinesterase activity and some of the FOB parameters at 1500 ppm. After 13 weeks of dosing, male forelimb and hindlimb grip strength was reduced at the top dose level. It was noted that there were no effects on plasma or erythrocyte cholinesterase activities in this dietary study. A NOAEL of 150 ppm was determined for both sexes (equivalent to 9.42 and 11.2 mg/kg bw/day, respectively).

### B.6.8 Other toxicological studies (AII 5.8)

#### B.6.8.1 Toxicity of the metabolites (IIA 5.8.1)

The following statement on the mammalian toxicology of methomyl oxime (IN-X1177) was provided by the company:

Methomyl, like other methyl carbamate insecticides, inhibits acetylcholinesterase (AChE) in the nervous system of insects and mammals. It exerts its biological activity by serving as a substrate for AChE which subsequently hydrolyses the carbamate ester from the parent molecule resulting in carbamylation and inhibition of the enzyme (Fukuto, 1990; O'Brien 1976). It is generally recognised that carbamate insecticides lose their biological activity upon cleavage of the carbamate moiety. Therefore, methomyl oxime, decarbamylated methomyl (IN-X1177), will not induce acetylcholinesterase inhibition as the carbamate moiety is absent.

A broad range of toxicity studies has been performed with methomyl in mammalian species (i.e., rats, mice, rabbits and dogs). These studies include acute, subacute, subchronic, and chronic/oncogenicity studies as well as studies which evaluated developmental and reproductive toxicity, mutagenicity, and neurotoxicity. The primary toxicological effect of methomyl is inhibition of AChE and is the endpoint upon which reference standards (i.e., ARfD, ADI, AOELs) for human health risk assessments have been proposed.

Based on metabolism studies conducted in rats, the cleavage of the carbamate ester to release methomyl oxime and the subsequent degradation to CO<sub>2</sub> has been proposed as

one of the major metabolic and elimination pathways (23% of the administered dose) for methomyl. Although methomyl oxime was not observed in the urine of methomyl-treated rats, CO<sub>2</sub> has been shown to be a major degradate of methomyl oxime when orally administered to rats (21.8% in the first 24 hours following dosing) (Huhtanen and Dorough, 1976). These results indicate that methomyl oxime is rapidly metabolised and is the major precursor in the formation of CO<sub>2</sub>. A similar metabolic pathway involving the metabolism of methomyl to methomyl oxime and subsequent degradation to CO<sub>2</sub> has been proposed for the non-human primate based on studies in Cynomolgus monkeys.

On the basis of the metabolism studies conducted with methomyl, it is reasonable to conclude that methomyl oxime was intrinsically evaluated in the broad array of toxicological studies conducted with the parent molecule. Moreover, methomyl oxime does not pose a risk of inducing AChE inhibition, which is the primary and most sensitive parameter for assessing methomyl-induced toxicity. Therefore, it has been concluded that the methomyl oxime metabolite does not pose a toxicological concern relative to parent methomyl.

### References:

Fukuto, T. R., 1990, Mechanism of Action of Organophosphorus and Carbamate Insecticides, Environmental Health Perspectives, 87:245-254.

Huhtanen, K., and Dorough, H.W., 1976, Isomerization and Beckmann rearrangement reactions in the metabolism of methomyl in rats. Pesticide Biochemistry and Physiology 6:571-583.

O'Brien, R.D., 1976, Acetylcholinesterase and its inhibition. In Insecticide Biochemistry and Physiology, ed. Wilkinson, C.F., Plenum Press, NY, pp. 271-296.

### 6.8.2 *In vitro* investigations into cholinesterase inhibition and reactivation

<b>Report</b>	Carakostas, M.C. (1987). Inhibition and regeneration kinetics for human and rat acetylcholinesterase exposed to methomyl - <i>in vitro</i> . Unpublished DuPont Report No.: HLR 379-88 (in-life phase: 1987 ).
<b>Test facility</b>	DuPont Haskell Laboratory, P.O. Box 50, Elkton Road, Newark, Delaware, 19714 USA
<b>Guidelines</b>	Not applicable
<b>Deviations from guidelines</b>	Not applicable
<b>GLP</b>	No
<b>Test substances</b>	DPX-X1179 Technical
<b>Batch</b>	X1179
<b>Purity</b>	98%
<b>Appearance</b>	White solid
<b>Acceptable</b>	Yes

### Materials and Methods

### Inhibition experiments

Human acetylcholinesterase (AChE), derived from erythrocytes, was purchased from the Sigma Chemical Company. Rat enzyme was prepared by haemolysing whole blood from male rats in 0.1% sodium carbonate and the haemolysate [containing both AChE and butyrylcholinesterase (BuChE), the latter being the principal enzyme present in plasma] was used in subsequent experiments.

Methomyl, in the concentration range of  $1.7\text{--}9.9 \times 10^{-6}$  M, was incubated with rat and human enzyme and cholinesterase activity determined using a Coulter DACOS-2 and a Cholinesterase ReagentSet (Boehringer Mannheim Diagnostics).  $IC_{50}$  data (molar concentration of methomyl that yields a 50% reduction in enzyme activity) was calculated using the following equation:

$$IC_{50} = 2.303 * (\log 100/50) \div (k_i * t)$$

Where  $k_i$  is the slope of the best-fit regression line of  $\log A_0$  (uninhibited cholinesterase activity) divided by  $A_t$  (cholinesterase activity) plotted against time, which was set at ten minutes.

### Regeneration experiments

The enzyme solution was incubated at 30°C for 10-15 minutes with enough methomyl to yield a 32-81% reduction in cholinesterase activity. The solution was then passed through a Sephadex G-25 column (PD-10 Pharmacia) and one-millilitre fractions were collected. Fractions 5 and 6, which contained the majority of enzyme activity, were further combined and incubated at 30°C. Timed serial samples were removed and analysed for cholinesterase activity. The activity data (the log percent of inhibition) was plotted against time and a slope of the best-fit regression line determined. The regeneration constant ( $k_3 = 2.303 \times \text{slope}$ ) and the terminal half-life ( $t_{0.5} = 0.69/k_3$ ) were determined.

### **Results**

Mean  $IC_{50}$  values for human ( $0.265 \times 10^{-5}$  M) and rat ( $1.56 \times 10^{-5}$  M) indicated that a 5.8-fold lower concentration of methomyl was required to reduce human AChE activity 50% compared to the rat acetyl/butyrylcholinesterase mixture.

Table 6.70. Rate constants and  $IC_{50}$  values

Run	Methomyl Concentration	$k_i$ (M/min)	$IC_{50}$ (M)
<b>Human acetylcholinesterase</b>			
A	$9.9 \times 10^{-6}$ M	$1.56 \times 10^4$	$0.444 \times 10^{-5}$
C	$9.9 \times 10^{-6}$ M	$2.52 \times 10^4$	$0.275 \times 10^{-5}$
X	$9.9 \times 10^{-6}$ M	$2.22 \times 10^4$	$0.312 \times 10^{-5}$
Y	$1.7 \times 10^{-6}$ M	$3.11 \times 10^4$	$0.223 \times 10^{-5}$
Z	$1.7 \times 10^{-6}$ M	$4.41 \times 10^4$	$0.157 \times 10^{-5}$
B	$5.0 \times 10^{-6}$ M	$3.93 \times 10^4$	$0.176 \times 10^{-5}$
Mean values (SD)		$2.96 \times 10^4$	$0.265 \times 10^{-5}$

		(1.07 x 10 <sup>4</sup> )	(0.106 x 10 <sup>-5</sup> )
<b>Rat acetyl/butyrylcholinesterase</b>			
1	9.9 x 10 <sup>-6</sup> M	4.16 x 10 <sup>3</sup>	1.67 x 10 <sup>-5</sup>
2	9.9 x 10 <sup>-6</sup> M	5.14 x 10 <sup>3</sup>	1.35 x 10 <sup>-5</sup>
5	9.9 x 10 <sup>-6</sup> M	3.60 x 10 <sup>3</sup>	1.93 x 10 <sup>-5</sup>
3	7.4 x 10 <sup>-6</sup> M	3.69 x 10 <sup>3</sup>	1.88 x 10 <sup>-5</sup>
4	7.4 x 10 <sup>-6</sup> M	7.00 x 10 <sup>3</sup>	9.90 x 10 <sup>-5</sup>
Mean values (SD)		4.73 x 10 <sup>3</sup> (1.41 x 10 <sup>3</sup> )	1.56 x 10 <sup>-5</sup> (0.39 x 10 <sup>-5</sup> )

The mean regeneration half-life ( $t_{0.5}$ ) was 38.0 and 26.6 minutes for human and rat AChE, respectively.

Table 6.71. Regeneration rate constants ( $k_3$ ) and half-life velocities ( $t_{0.5}$ )

Run	$k_3 \times 10^{-2}/\text{min}$	$t_{0.5} (\text{min})$
<b>Human acetylcholinesterase</b>		
1	1.89	36.40
2	1.51	45.60
3	1.76	39.20
4	2.24	30.80
Mean values (SD)	1.85(0.30)	38.00 (6.15)
<b>Rat acetyl/butyrylcholinesterase</b>		
1	2.26	30.53
2	3.05	22.59
3	2.47	27.95
4	2.71	25.49
Mean values (SD)	2.62 (0.34)	26.64 (3.39)

## Conclusions

The inhibition rate constants ( $k_i$ ),  $IC_{50}$  concentration values, regeneration rate constants ( $k_3$ ), and the regeneration half-lives ( $t_{0.5}$ ) were determined *in vitro* for human AChE and rat red blood cell whole blood (AChE and BuChE) cholinesterase. The concentration of methomyl required to produce a 50% reduction in human enzyme activity ( $0.265 \times 10^{-5}$  M) was approximately 6-fold lower compared to the rat ( $1.56 \times 10^{-5}$  M). The regeneration half-life ( $t_{0.5}$ ) was slightly longer for human AChE (38.0 minutes) compared to rat AChE/BuChE (26.6 minutes). However, interpretation of the results is confounded by the fact that the human and rat enzyme samples were not comparable. The results are also questionable as the range of methomyl concentrations tested were all below the estimated  $IC_{50}$  values and thus would require extrapolation outside the tested range.

(Carakostas, 1987)

6.8.3 *In vivo* investigations into cholinesterase inhibition and reversibility in rats

a)

<b>Report</b>	Filliben, T. (1996). Acute dietary toxicity study for cholinesterase inhibition with DPX-X1179 in male rats. DuPont Report No.: HLR 861-96 (in-life phase: June 1997).
<b>Test facility</b>	DuPont Haskell Laboratory, P.O. Box 50, Elkton Road, Newark, Delaware, 19714 USA
<b>Guidelines</b>	Not available
<b>Deviations from guidelines</b>	Not applicable
<b>GLP</b>	
<b>Test substances</b>	DPX-X1179 Technical
<b>Batch</b>	X1179-394
<b>Purity</b>	98.4%
<b>Appearance</b>	White solid
<b>Acceptable</b>	Yes

**Materials and Methods**

The objective of this study was to investigate the acute dietary toxicity of methomyl when incorporated into diet and fed for a 2-hour period. Approximately 10 grams of diet containing 0, 30, 60, 120, or 360 ppm of methomyl were presented for consumption to young adult male Crl:CD (SD)BR rats (10/dose) for approximately 2 hours each day. Rats were pre-conditioned to eat within a 2-hour time frame during an 18-day pre-test period. Dose selection was based on pilot studies.

Clinical observations, body weight and food consumption were recorded. Approximately 1 hour after the end of the feeding period, a modified functional observational battery (MFOB) was conducted on each rat (grip strength and foot splay were not evaluated). Following the MFOB, blood was collected from the orbital sinus of each rat for plasma and RBC activity determinations. Rats were sacrificed and brains were collected for determination of brain cholinesterase activity. Blood samples were collected from the orbital sinus and analysed on the day of collection. The brain tissue was stored on ice until frozen at -70°C and analysed at a later date. All cholinesterase determinations were carried out using a Hitachi 717 analyser and Boehringer Mannheim reagents (based on the Ellman method).

**Results**

Analysis confirmed that the concentration, homogeneity and stability of the test substance in the diet were acceptable. The mean daily intake values were 0, 0.953, 1.88, 3.74 and 9.98 mg/kg bw at 0, 30, 60, 120 and 360 ppm, respectively.

No deaths occurred during the study. There were no clinical signs of toxicity during the exposure period. During the MFOB, no significant findings were observed during the in-cage observations. During the free-roaming portion of the MFOB, a statistically significant increase in the incidence of low arousal was noted in the 360 ppm group (Table 6.72). During the manipulations period of the MFOB assessment, males in the 360 ppm group had significantly higher incidence of no reaction to approach-and-touch stimulus; and in the 60, 120, and 360 ppm groups, there was a significantly higher incidence of rats with no reaction to tail-pinch stimulus.

Table 6.72. Mean body weight and food consumption data and incidences of findings in the MFOB (n = 10/dose)

Dose (ppm)	0	30	60	120	360
Body weight on test day (g) (standard deviation)	314.7 (18.3)	312.8 (15.4)	313.1 (16.8)	313.6 (16.1)	311.8 (17.5)
Food consumption (standard deviation)	10.1 (0.3)	9.9 (0.2)	9.8 (0.2)	9.8 (0.2)	8.6* (1.4)
Low arousal	0	1	2	2	7*
No reaction to approach and touch	1	4	6	3	7*
No reaction to tail-pinch	0	1	3*	5*	10*

Key: a) In cage observations at 360 ppm included 1/10 males with palpebral closure/ptosis and 1/10 with curled up posture (asleep or sitting with head hung low).

The cholinesterase determinations are presented in Table 6.73.

Table. 6.73. Summary of the cholinesterase determinations

Dose (ppm)	0	30	60	120	360
Plasma cholinesterase (U/l)	408 (48)	429 (68)	420 (73)	340 (57)* [↓ 17%]	257 (40)* [↓ 37%]
Erythrocyte cholinesterase (U/l)	1350 (256)	1176 (200) [↓ 13%]	1070 (342) [↓ 21%]	922 (390)* [↓ 32%]	674 (210)* [↓ 50%]
Brain cholinesterase (U/g)	11.6 (0.7)	10.8 (0.9) [↓ 7%]	10.8 (0.3) [↓ 7%]	8.3 (1.1)* [↓ 29%]	6.2 (0.5)* [↓ 47%]

Plasma, RBC and brain ChE activities were significantly decreased in the 120 and 360 ppm groups. The mean values for these parameters exhibited a dose-response relationship and the changes were considered test substance-related and biologically adverse. At 60 ppm the inhibition was >20 % but was not statistically significant according to the company but had a p value of <0.03 in an unpaired single sided student's t-test.

## Conclusions

The NOAEL for this study was 30 ppm (1 mg/kg bw) in male rats based on the increased incidence of a diminished tail-pinch response and >20% inhibition of erythrocyte cholinesterase at 60 ppm.

(Filliben, 1996)

b)

<b>Report</b>	Sherman, H. (1964); Ten-dose subacute oral test. Unpublished DuPont Report No. HLR 100-64. (in-life phase: 1964).
<b>Test facility</b>	DuPont Haskell Laboratory, P.O. Box 50, Elkton Road, Newark, Delaware, 19714 USA
<b>Guidelines</b>	Not applicable
<b>Deviations from guidelines</b>	Not applicable
<b>GLP</b>	No
<b>Test substances</b>	DPX-X1179 Technical
<b>Batch</b>	X1179-1
<b>Purity</b>	Not specified
<b>Appearance</b>	White solid
<b>Acceptable</b>	No (not acceptable for regulatory purposes on a stand-alone basis)

## Materials and Methods

A ten-dose oral subacute test was performed with methomyl. The test substance, as a 0.1% suspension in peanut oil, was administered by intragastric intubation at a dose level of 5.1 mg/kg bw/day to six young adult ChR-CD male rats. The animals were gavaged five times a week for 2 weeks. Concurrent control groups were gavaged with peanut oil. Three control and 3 test rats were sacrificed approximately 4 hours after the last dose. The remaining three control and three test rats were sacrificed 14 days after the last dose. Plasma cholinesterase was measured (no details of methodology) in cardiac blood taken 4 hours and 14 days after the tenth dose. A pathological exam was performed on all test animals. The following tissues were examined microscopically: heart, lung, stomach, duodenum, liver, pancreas, kidney, spleen, thymus, bone marrow, pituitary, adrenal, testis, brain, skeletal muscle and nerve.

## Results

There was no test substance-related mortality observed during the course of the study. Clinical signs of toxicity during the dosing period included mild fasciculations, profuse salivation, pallor, chewing, “yawning” motions and irritability. Plasma cholinesterase activity measurements conducted on cardiac blood taken from rats 4 hours and 14 days after the tenth dose were not significantly different from controls. There were no test substance-related histopathologic changes observed.

## Conclusions

Methomyl caused clinical signs of toxicity in rats administered 10 doses of 5.1 mg/kg bw/day over a 2-week period.

(Sherman, 1964)

c)

<b>Report</b>	Malley, L.A. (1997). Reversibility study with carbamate insecticides in rats. Unpublished DuPont Report No.: HL-1997-00641 (in-life phase: June 1997).
<b>Test facility</b>	DuPont Haskell Laboratory, P.O. Box 50, Elkton Road, Newark, Delaware, 19714 USA
<b>Guidelines</b>	Not available
<b>Deviations from guidelines</b>	Not applicable
<b>GLP</b>	
<b>Test substances</b>	DPX-X1179 Technical
<b>Batch</b>	X1179-512
<b>Purity</b>	98.6%
<b>Appearance</b>	White solid
<b>Acceptable</b>	Yes

### Materials and Methods

The objective of this study was to determine the length of time needed for recovery from inhibition of cholinesterase activity. Groups of Sprague Dawley rats (40/sex/dose) were orally administered (by gavage) methomyl technical in deionised water at concentrations of 0 or 3 mg/kg bw. Dose selection and the time-to-recovery time points were based on pilot studies. Body weights were taken just prior to treatment in order to obtain dosing volumes for the rats. Clinical signs of toxicity were recorded for all animals at 30 minutes post dosing. Cholinesterase (ChE) activity (RBC, plasma, and whole brain) was assessed in 10 rats/sex/group at 30 minutes and 2, 3 and 4 hours post dosing. Blood samples were kept on ice and analysed on the day of collection. The brain tissue was stored on ice until frozen at -70°C and analysed at a later date. All cholinesterase determinations were carried out using a Hitachi 717 analyser and Boehringer Mannheim reagents (based on the Ellman method).

### Results

Analysis confirmed that the concentration of the test substance in the dosing solutions was acceptable for the main study.

Test substance-related mortality did not occur during the course of this study. Clinical signs indicative of ChE inhibition were noted within 30 minutes of dosing (predominant sign was tremors; lacrimation was noted in one male); recovery occurred within 2 hours post dosing (Table 6.74).

WARNING: This document forms part of an EC evaluation data package and should not be used for registration purposes. Registration must not be granted on the basis of this document.



Table 6.74. Cholinesterase determinations

Dose (mg/kg bw)		0	3
Plasma cholinesterase (U/l)			
Males (n= 10)	0.5 hours	421 (50) <sup>a</sup>	306 (55)* [↓ 27%]
	2.0 hours	437 (63)	356 (53)* [↓ 19%]
	3.0 hours	449 (69)	449 (73)
	4.0 hours	458 (55)	399 (38)* [↓ 13%]
Females (n= 10)	0.5 hours	784 (279)	703 (222)* [↓ 10%]
	2.0 hours	835 (216)	782 (191)
	3.0 hours	857 (363)	884 (227)
	4.0 hours	856 (301)	738 (202)
Erythrocyte cholinesterase (U/l)			
Males (n= 10)	0.5 hours	2074 (393)	920 (231)* [↓ 56%]
	2.0 hours	2294 (415)	1754 (267)* [↓ 24%]
	3.0 hours	2042 (400)	1990 (485)
	4.0 hours	2126 (432)	2082 (304)
Females (n= 10)	0.5 hours	2342 (375)	1374 (188)* [↓ 41 %]
	2.0 hours	2062 (500)	2084 (291)
	3.0 hours	2250 (515)	2146 (272)
	4.0 hours	2334 (276)	2430 (271)
Brain cholinesterase (U/l)			
Males (n= 10)	0.5 hours	12.30 (0.86)	6.65 (0.92)* [↓ 46%]
	2.0 hours	11.98 (0.47)	10.06 (0.71)* [↓ 16%]
	3.0 hours	11.96 (0.41)	11.06 (0.46)* [↓ 8%]
	4.0 hours	11.83 (0.33)	11.76 (0.37)
Females (n= 10)	0.5 hours	12.12 (0.52)	7.37 (0.54)* [↓ 39%]
	2.0 hours	12.11 (0.44)	11.17 (0.70)* [↓ 8%]
	3.0 hours	12.46 (0.58)	11.43 (0.58)* [↓ 8%]
	4.0 hours	10.09 (0.66)	12.07 (0.44)

Key a) Standard deviation.

Statistically significant decreases in plasma, RBC, and brain ChE activities occurred at 30 minutes after dosing. By 2 hours post dosing, males had minimal residual inhibition of blood and brain cholinesterase activity; recovery was complete by 3 hours. In females recovery was complete by 2 hours.

## Conclusions

A dose of 3 mg/kg bw methomyl caused clinical signs (tremors) indicative of cholinesterase inhibition and RBC, plasma, and brain cholinesterase inhibition within 30 minutes of dosing. Recovery of clinical signs and cholinesterase inhibition was complete by 2 hours post dosing in females and by 3 hours in males

(Malley, 1997)

## B.6.8.4 Human volunteer studies

<b>Report</b>	McFarlane, P., Sanderson, J.B., Freestone, S. (1998). A randomised double-blind ascending oral dose study with methomyl to establish a no adverse effect level. Unpublished DuPont Report No.: HLO-1998-00969 (2 volumes) (in-life phase: November 1997 to December 1997).
<b>Test facility</b>	Inveresk Research International (IRI), Limited, Research Park (Scotland), Tranent, Scotland, U.K.
<b>Guidelines</b>	None available.
<b>Deviations from guidelines</b>	Not applicable
<b>GLP</b>	Yes (UK authorities).
<b>Test substance</b>	DPX-X1179 Technical.
<b>Batch</b>	Lot T101397-00
<b>Purity</b>	89%
<b>Appearance</b>	White solid
<b>Acceptable</b>	Yes

**Materials and methods**

The following study was performed according to the declaration of Helsinki (as amended) on bio-medical studies in volunteers, involved prior informed consent and prior approval by an ethics committee.

A single oral dose of methomyl was given to 19 healthy male volunteers aged 18-40 years, to determine the no adverse effect level. A methomyl formulation (methomyl 90SP containing 90% methomyl) and its matching placebo (the formulation ingredient was hydrated silicon) were each administered as a capsule at doses of 0, 0.1, 0.2, or 0.3 mg/kg bw. The volunteers (4 in the control group; 5 in the treated groups) were observed for a 24-hour period and one follow-up visit 7 ( $\pm 2$ ) days post dosing. The study was conducted as a double-blinded ascending dose escalation clinical trial in which the dose increased for a subsequent dose group only if the results for all lower groups indicated it was safe to do so.

Parameters evaluated during the study included physical examination, vital signs, oral temperature, 12 lead electrocardiogram (ECG), and continuous ECG monitoring. Pupillary size and saliva collection were measured as sensitive indicators for cholinergic responses. Plasma and RBC cholinesterase (ChE) activities were measured at multiple time points to monitor inhibition and return to baseline levels. Each subject's ECG was monitored continuously from -30 min through 3 hours post dosing, and 12 lead ECGs were recorded at screening, -30 min, 30 min, and 1, 2 and 24 hours post dosing. Vital signs (blood pressure, heart rate, clinical signs) were taken at screening, -16 hours (on admission to clinic), -30 min, 1, 2, 3, 4, 8 and 24 hours post dose. Blood samples were collected for clinical chemistry and haematology evaluations at screening, -30 min and 24 hours post dose. Urinalysis was performed at screening and 24 hours post dose. Oral temperature was recorded at screening, -16 hours, -30 min, 2, 4 and 24 hours post dose. RBC and plasma ChE samples were collected and immediately frozen at screening, day -2, -16 h, -30 min, every 15 min for the first 2 hours after dosing, 3, 4, 6, 8, 12 and 24 hours post dosing and again one week later. Pupil size was measured at -16 hours, -30 min, 1, 2, 3, 4, 8 and 24 hours post dose. The quantity of saliva secreted within a 5-minute period was measured at -16 hours, -30 min, 1, 2, 3, 4, 8 and 24 hours post dose.

## Results

The concentration of methomyl in the capsules was verified by analysis. The dissolution profile of the methomyl capsules was evaluated in a tank containing 900 ml of 0.01M HCl at 37°C. Within 15 minutes, the mean percent dissolved was >90% and complete dissolution had occurred with 60 minutes.

The dosing schedule is presented in Table 6.75.

Table 6.75. The actual dosing schedule (18 white males plus 1 Asian)

Schedule	Placebo	0.1 mg/kg bw	0.2 mg/kg bw	0.3 mg/kg bw
Session 1/No of subjects	1 (Subject 2)	1 (Subject 1 <sup>#</sup> )	-	-
Session 2/No of subjects	1 (Subject 4)	4 (Subjects 3, 6, 7 & 8)	-	1 (Subject 5 <sup>##</sup> )
Session 3/No of subjects	1 (Subject 12)	-	-	4 (Subjects 9, 11, 13 & 14 <sup>###</sup> )
Session 4/No of subjects 15-20	1 (Subject 19)	-	5 (Subjects 15, 16 <sup>###</sup> , 17, 18 & 20)	-
Total subjects = 19	4	5	5	5

Key: a) # Subject No 1 exhibited >40% inhibition of red blood cell cholinesterase at 8 hours post dose. This reduction was considered a spurious laboratory result, since in other cases reduction in activity had occurred much earlier and recovered by 6 hours post dose. b) ## Subject No 5 exhibited >40% inhibition of red blood cell cholinesterase and reported headache (that did not require treatment) 1.75 hours after dosing which was over within one hour. c) ### Subject Nos 14 & 16 exhibited >40% inhibition of red blood cell cholinesterase. d) Subject No 10 was not dosed.

No adverse effects requiring treatment with atropine were observed during the study. Subject No 1 (0.1 mg/kg bw) reported the onset of insomnia approximately 1.5 days after dosing which lasted for 2 days. He also reported an onset of stomach cramps and diarrhoea approximately 1.5 days after dosing which lasted for 3 days. None of these symptoms were considered to be related to treatment because of the late onset in relation to dosing. Subject No 5 (0.3 mg/kg bw) reported headache 1.75 hours after dosing. The headache lasted for approximately 1 hour. The subject also reported somnolence and lethargy with an onset of approximately 3 days after dosing and a duration of 1 day. This latter event was not considered to be related to methomyl administration.

There were no treatment related effects on ECG, heart rate, pulse, blood pressure, respiratory rate, body temperature, haematology parameters, clinical chemistry parameters (excluding cholinesterase activities), urinalysis, or pupillametry.

The percentage change of plasma and red blood cell cholinesterase activity from the base line are presented in Tables 6.76 & 6.77, respectively.

Table 6.76. Mean inhibition of plasma cholinesterase activity in human volunteers after a single oral dose of methomyl (capsules)

Time (hours)	Percent change from baseline <sup>a</sup>			
	Placebo (n = 4)	0.1 mg/kg bw (n = 5)	0.2 mg/kg bw (n = 5)	0.3 mg/kg bw (n = 5)
0.25	-1.3 (0.7) <sup>b</sup> [-7 to 5] <sup>c</sup>	-3.1 (3.6) [-9 to 1]	-2.5 (2.8) [-6 to 0]	-9.8* (2.0) [-13 to -8]
0.5	-2.9 (4.8) [-7 to 4]	-6.6 (3.9) [-12 to -3]	-7.9 (3.7) [-13 to -4]	-15.9** (2.2) [-18 to -13]
0.75	-1.4 (3.8) [-6 to 2]	-5.6 (2.1) [-8 to -2]	-11.5** (5.5) [-16 to -2]	-21.1** (4.9) [-28 to -15]
1	-0.3 (3.0) [-4 to 2]	-4.5 (3.4) [-8 to 1]	-11.0** (4.2) [-15 to -5]	-16.6** (2.2) [-19 to -14]
1.25	-0.8 (4.7) [-5 to 4]	-2.9 (5.7) [-7 to 6]	-13.3** (5.1) [-19 to -7]	-19.7** (2.9) [-24 to -17]
1.5	-2.4 (5.7) [-8 to 3]	-5.9 (3.9) [-12 to -2]	-12.9** (2.5) [-16 to -9]	-14.8** (4.2) [-22 to -10]
1.75	-2.7 (4.8) [-9 to 2]	-5.6 (1.8) [-8 to -3]	-13.5** (2.0) [-17 to -11]	-15.9** (3.8) [-19 to -10]
2	-1.0 (3.8) [-4 to 3]	-7.2 (3.6) [-13 to -5]	-10.3* (3.3) [-16 to -7]	-14.1** (5.6) [-22 to -9]
3	-1.3 (4.8) [-7 to 5]	-1.1 (4.7) [-6 to 6]	-5.0 (4.6) [-12 to -1]	-10.9* (3.0) [-16 to -7]
4	0.0 (7.3) [-9 to 9]	-2.5 (2.1) [-5 to 1]	-1.3 (3.2) [-5 to 3]	-8.1* (3.8) [-13 to -2]
6	0.6 (6.4) [-7 to 8]	-0.2 (3.8) [-4 to 4]	-2.1 (5.0) [-9 to 5]	0.2 (3.1) [-4 to 4]
8	0.4 (8.4) [-8 to 11]	-0.5 (3.8) [-6 to 4]	0.6 (4.1) [-4 to 6]	-0.9 (2.4) [-4 to 1]
12	-3.0 (8.5) [-13 to 6]	-0.8 (3.9) [-7 to 3]	-0.7 (1.7) [-2 to 2]	8.4 (17.2) [-2 to 39]
24	1.3 (8.2) [-11 to 8]	2.3 (4.0) [-4 to 6]	0.5 (5.3) [-3 to 10]	3.4 (7.8) [-5 to 13]

Key: a) Baseline = mean of values at -16 and -30 hours. b) Standard deviation. c) Range. d) \* Statistically significant ( $p < 0.05$ ). e) \*\* Statistically significant ( $p < 0.01$ ).

Statistically significant depressions in RBC and plasma cholinesterase activity were evident at doses of 0.2 and 0.3 mg/kg bw. There was a single occurrence of a mild headache at 0.3 mg/kg bw and quantitatively increased salivation at 0.2 and 0.3 mg/kg bw. Increased salivation was subtle, as this increase was not clinically observed but was only detected by measuring the weight of secretion absorbed with dental rolls over a 5 minute period. Plasma and RBC cholinesterase were significantly reduced in the 0.2 mg/kg bw group from 45 minutes up to and including the 2 hour time point and in the 0.3 mg/kg bw group from 15 minutes posting dosing up to and including the 4 hour time point. At 0.1 mg/kg bw/day, the mean red blood cell cholinesterase activity was depressed by 19% at 1.25 hours post dosing (Table 6.77). The individual red blood cell cholinesterase determinations are presented in Table 6.78.

Table 6.77. Mean inhibition of red blood cell cholinesterase activity in human volunteers after a single oral dose of methomyl (capsules)

Time (hours)	Percent change from baseline <sup>a</sup>			
	Placebo (n = 4)	0.1 mg/kg bw (n = 5)	0.2 mg/kg bw (n = 5)	0.3 mg/kg bw (n = 5)
0.25	5.8 (9.8) <sup>b</sup> [-3 to 20] <sup>c</sup>	3.1 (17.5) [-21 to 27]	-1.2 (9.3) [-11 to 13]	-18.6** (12.4) [-32 to 1]
0.5	-1.8 (5.6) [-10 to 4]	-9.2 (13.0) [-28 to 5]	-12.4 (5.6) [-19 to -5]	-32.0** (3.6) [-35 to -26]
0.75	-2.9 (14.9) [-16 to 18]	-2.4 (12.1) [-12 to 15]	-20.0* (14.1) [-34 to 4]	-35.3** (10.4) [-47 to -21]
1	-4.0 (17.3) [-30 to 8]	-14.6 (11.6) [-31 to 0]	-24.7** (9.4) [-39 to -15]	-27.3** (7.5) [-38 to -17]
1.25	-4.3 (5.8) [-8 to 4]	<sup>d</sup> -19.0 (9.3) [-31 to 7]	-27.6** (10.7) [-41 to -18]	-26.8** (7.3) [-34 to -15]
1.5	-0.3 (5.6) [-4 to 8]	-10.6 (10.6) [-20 to 4]	-27.9** (7.4) [-39 to -21]	-23.2** (7.4) [-31 to -15]
1.75	1.8 (5.9) [-4 to 10]	-3.7 (11.5) [-21 to 9]	-22.2** (4.9) [-28 to -16]	-22.4** (10.7) [-37 to -12]
2	5.8 (14.0) [-4 to 26]	-8.9 (11.2) [-24 to 1]	-16.2* (5.6) [-23 to -10]	-16.0** (8.6) [-26 to -8]
3	12.1 (12.7) [2 to 28]	-2.1 (12.5) [-21 to 10]	-1.3 (7.4) [-7 to 12]	-12.9** (16.5) [-38 to 3]
4	11.4 (10.1) [3 to 24]	5.0 (12.3) [-7 to 21]	-2.3 (8.8) [-12 to 11]	-5.0* (8.5) [-16 to 5]
6	6.2 (12.8) [-9 to 20]	-5.9 (16.3) [-17 to 22]	14.8 (6.4) [9 to 25]	-2.0 (6.7) [-12 to 5]
8	-4.4 (14.8) <sup>e</sup> [-21 to 14]	-8.7 (25.1) [-44 to 16]	9.0 (5.6) [3 to 17]	-0.5 (4.8) [-7 to 5]
12	-5.7 (16.8) <sup>f</sup> [-27 to 14]	-15.3 (17.0) [-34 to 11]	14.4 (8.5) [5-28]	-5.6 (11.7) [-22 to 4]
24	-4.9 (15.9) <sup>g</sup> [-27 to 10]	-3.7 (20.0) [-21 to 29]	10.0 (10.8) [0-28]	-1.4 (5.9) [-9 to 4]

Key: a) Baseline = mean of values at -16 and -30 hours. b) Standard deviation. c) Range. d) p value = 0.072 (Student 't'-test), no clear indications of a treatment related effect at this dose level at other time points or in plasma cholinesterase activities at this dose level. e) Low value in Subject No 2. f) Low value in Subject No 4. g) \* Statistically significant (p < 0.05). h) \*\* Statistically significant (p < 0.01).

In the 0.1 mg/kg bw group, four of the subjects had red blood cell cholinesterase depression (>20%) as did at least 1 of the placebo subjects. The red blood cell cholinesterase activity in the remaining subject was greater than the baseline for all the post-dosing time point except one. The baseline for this individual was noticeably lower than the other members of the group. The data for these four individuals with erythrocyte cholinesterase depression (>20%) tended to have a biphasic pattern (peak depression between 0.5-2 hours & 6-24 hours).

Table 6.78. Individual values for the 0.1 mg/kg bw dose group

Time (hours)	Red blood cell cholinesterase activity (IU/l)				
	Subject No 1	Subject No 3	Subject No 6	Subject No 7	Subject No 8
Screening	8219	8277	6683	8813	8462
-16	11067	11721	7353	10215	7488
-30	13059 <sup>b</sup>	10662	7806	9465	10734
<sup>a</sup> Baseline	12063	11192	7580	9840	9111
0.25	11577 (↓ 4%)	8889 (↓ 21%)	9606	10170	10044
0.5	11052 (↓ 8%)	9615 (↓ 14%)	7956	7056 (↓ 28%)	9093
0.75	13833 <sup>b</sup>	10161 (↓ 9%)	8034	8616 (↓ 12%)	8085 (↓ 11%)
1	9756 (↓ 19%)	9699 (↓ 13%)	7560	6753 (↓ 31%)	8295 (↓ 9%)
1.25	8154 (↓ 32%)	9378 (↓ 16%)	7056 (↓ 7%)	8136 (↓ 17%)	7074 (↓ 22%)
1.5	9756 (↓ 19%)	10809	7887	7833 (↓ 20%)	7824 (↓ 14%)
1.75	9471 (↓ 21%)	11196	8292	9867	8511
2	9171 (↓ 24%)	11157	7680	8115 (↓ 17%)	8739
3	11685	12321	8169	7731	8751
4	11178	13521 <sup>b</sup>	8514	10443	8464
6	10644 (↓ 12%)	9234 (↓ 17%)	9261	8241 (↓ 16%)	8553
8	6810 (↓ 44%)	8739 (↓ 22%)	8796	9042 (↓ 8%)	10374
12	7911 (↓ 34%)	8697 (↓ 22%)	8436	8679 (↓ 12%)	7338 (↓ 19%)
24	9576 (↓ 21%)	9507 (↓ 15%)	9813	8664 (↓ 12%)	9060
Post study	12339	11616	10323	11784	10293

Key: a) Baseline = mean of values at -16 and -30 hours. b) Above normal range.

WARNING: This document forms part of an EC evaluation data package and should not be read in isolation. Registration must be granted on the basis of this document.

Table 6.79. Individual values for the placebo subjects

Time (hours)	Red blood cell cholinesterase activity (IU/l)			
	Subject No 2	Subject No 4	Subject No 12	Subject No 19
Screening	7785	8861	8110	8190
-16	9267	11487	11256	13167 <sup>b</sup>
-30	9159	12252	12222	13365 <sup>b</sup>
<sup>a</sup> Baseline	9213	11870	11739	13266 <sup>b</sup>
0.25	11031	11484	12174	13680 <sup>b</sup>
0.5	8316	11811	12153	13209 <sup>b</sup>
0.75	10860	10005	10428	12906 <sup>b</sup>
1	9951	8355 (↓ 30%)	12318	13365 <sup>b</sup>
1.25	8601	10902	10953	13854 <sup>b</sup>
1.5	9957	11562	11418	12744 <sup>b</sup>
1.75	10113	11694	11319	13608 <sup>b</sup>
2	11646	12144	11274	13080 <sup>b</sup>
3	11814	12135	13635 <sup>b</sup>	13482 <sup>b</sup>
4	11409	12270	13527 <sup>b</sup>	13659 <sup>b</sup>
6	10317	10767	11970	15948 <sup>b</sup>
8	7290 (↓ 21%)	10740	11598	15147 <sup>b</sup>
12	9009	8637 (↓ 27%)	10953	15060 <sup>b</sup>
24	6690 (↓ 27%)	11517	11841	14574 <sup>b</sup>
Post study	10215	13884 <sup>b</sup>	12900 <sup>b</sup>	15183 <sup>b</sup>

Key: a) Baseline = mean of values at -16 and -30 hours. b) Above normal range.

## Conclusions

Significant depressions in plasma and RBC cholinesterase were observed in human volunteers following methomyl doses of 0.2 and 0.3 mg/kg bw. At the LOAEL, 0.2 mg/kg bw, cholinesterase activities returned to baseline within 3 hours post dosing. Under the conditions of this study, the NOAEL was 0.1 mg/kg bw for male volunteers. However, it should be noted that group size is small and there is wide variation in the cholinesterase values in the placebo (8-24 hours post dosing in two subjects) and the 0.1 mg/kg bw/day (one subject) groups.

### B.6.8.5 Human poisoning incidents

In a recent report, an outbreak of gastrointestinal symptoms was associated with the consumption of food seasoned with methomyl-contaminated salt (n = 107). The clinical symptoms reported included nausea (95%), dizziness (72%), abdominal cramps (58%), headache (52%), vomiting (51%), chills (48%) and diarrhoea (46%). The onset of symptoms occurred within two hours of eating and the median duration of symptoms was 6 hours. Testing of food and water samples for bacterial pathogens and other contaminants (including metals, mycotoxins, organophosphates and carbamates) were negative. Methomyl was identified in a sample of vomitus (20 ppm), in salt taken from containers (mean = 5600 ppm) and on the stove top (mean = 1425 ppm). The oral toxic dose causing symptoms in 50% of those exposed to methomyl was estimated to be 0.15 mg/kg bw (estimated range 0.09-0.31 mg/kg bw).

(Buchholz *et al*, 2002)

#### B.6.8.6. Summary

The *in vitro* assays comparing human AChE with rat whole blood (AChE and BuChE) cholinesterase showed that the concentration of methomyl required to produce a 50% reduction in human enzyme activity ( $0.265 \times 10^{-5}$  M) was approximately 6-fold lower compared to the rat ( $1.56 \times 10^{-5}$  M). The regeneration half-life ( $t_{0.5}$ ) was slightly longer for human AChE (38.0 minutes) compared to rat AChE/BuChE (26.6 minutes). However, interpretation of the results is confounded by the fact that the human and rat enzyme samples were not comparable. The results are also questionable as the range of methomyl concentrations tested were all below the estimated IC50 values and thus would require extrapolation outside the tested range.

In the acute dietary study, male rats pre-conditioned to eat within a 2-hour time frame were administered methomyl incorporated into diet. Following this bolus dietary dosing, there were significantly higher incidences of no reaction to approach-and-touch stimulus and no reaction to tail-pinch stimulus (grip strength was not evaluated). Significant reductions in plasma, erythrocyte and brain cholinesterase activities were also evident in this acute dietary study. In the short-term oral study (gavage), clinical signs typical of cholinesterase inhibition were seen shortly after dosing.

The reversibility study in rats showed that the cholinesterase inhibition and clinical signs peaked within 30 minutes of dosing. Both the cholinesterase levels and clinical signs had returned to control levels with 2-3 hours post dosing.

In the human volunteer study, there were statistically significant depressions in RBC cholinesterase activity at doses of 0.2 (0.75-1.75 post dosing) and 0.3 mg/kg bw (0.5-1.75 hours post dosing), a single occurrence of a mild headache at a dose of 0.3 mg/kg bw, and quantitatively increased salivation at 0.2 and 0.3 mg/kg bw. Red Blood cholinesterase activity was depressed by 19% at 0.1 mg/kg bw at 1.25 hour post dosing (<20% cut off). Plasma cholinesterase activity was depressed at 0.2 mg/kg bw and above.

Clinical symptoms have been reported in humans at an estimated acute oral dose level of 0.15 mg/kg bw (estimated range 0.09-0.31 mg/kg bw) in an epidemiology study.

#### B.6.9 Medical data

The information in this section was provided by the company and has not been evaluated by the rapporteur.

##### B.6.9.1 Medical surveillance on manufacturing plant personnel (AII 5.9.1)

Methomyl-containing products have been handled and manufactured at [REDACTED] for more than 20 years. All workers undergo an annual health check and those with potential exposure to methomyl undergo regular blood testing for cholinesterase activity before, during, and at the end of a production cycle. To prevent exposure of plant personnel to methomyl, periodic evaluations of process equipment are routinely undertaken. In addition air monitoring for dust and solvent is conducted at the most workplaces as a part of preventive measures. Risk of inhalation of methomyl is very well managed and minimised by wearing of respiratory protective equipment. To



minimise dermal exposure, protective clothing like Tyvek® coverall and nitrile gloves are worn. At [REDACTED] fume hoods have been installed at in the work areas where methomyl-containing material is loaded for manufacture and local air monitoring which is carried out on a routine basis, has shown that the AEL is not exceeded. The acceptable worker exposure limit (AEL) for methomyl is 2.5 mg/m<sup>3</sup> (8-hours time weighted average). There are procedures in place to ensure that in the event of an accidental exposure to methomyl appropriate medical treatment would be given and the accident recorded.

#### **B.6.9.2 Direct observation, e.g., clinical cases and poisoning incidents**

A search covering the time period from 1967 to present on available reports and documentation from the open literature relating to clinical cases and poisoning incidents was undertaken. The following databases were searched for clinical cases or poisoning incidents related to the agricultural use of methomyl-containing plant protection products on September 4, 2001:

- Toxicology Literature Online Databank (TOXLINE), a database of the National Library of Medicine's TOXNET system
- Scientific Technical Network, a network of over 1000 databases covering disciplines from the biosciences to engineering

The search parameters were methomyl, Metomil, Mesonil, Lannate®, epidemiology, human health, health effects, clinical poison (poisoning), incidence, incident, overexposure, poison, poisons, poisoned, poisoning, poisoning incident, poisoning incidence(s).

The literature search indicated several reports incidents or accidents related to the use of methomyl in agriculture. The reported casualties were all connected to the lack of safety precautions, mainly lack of protective clothing. Reports found in the open literature confirm in general the validity of extrapolating from animal to human data. In addition, results of a human volunteer study summarised (in section B.6.8.3) show that no other adverse toxic effects observed in humans than those described in the animal studies.

#### **B.6.9.3 Observations on exposure of the general population (AII 5.9.3)**

Exposure of the general population is expected to be very low and should not constitute a health hazard. Practical observations indicate that methomyl does not give rise to long-lasting adverse effects. Neither data related to exposure of the general public to methomyl nor epidemiological studies were available at the point when evaluation of this application began. However, an epidemiology study has subsequently become available: see Section B.6.8.5.

#### **B.6.9.4 Diagnosis of poisoning (AII 5.9.4)**

Poisoning due to overexposure to methomyl is related to inhibition of cholinesterase activity. Acute poisoning may arise very quickly, sometimes within minutes, and may encompass one or more different symptoms. Malaise, muscle weakness, dizziness,

and sweating are commonly reported early symptoms. Headache, excessive salivation, nausea, vomiting, abdominal pain, and diarrhoea are often prominent.

In severe cases of poisoning, dyspnea, bronchospasm, and chest tightness may result in pulmonary oedema. Blurred vision, muscle twitching and spasms may occur. Miosis, incoordination, and slurred speech are reported. Bradycardia occurs infrequently. Severe neurological manifestations, including convulsions and coma are less commonly observed than with organophosphate poisoning.

Long-term overexposure of laboratory animals to methomyl causes non-specific effects such as weight loss and mild transient changes in haematology parameters. Methomyl was not oncogenic in laboratory animals.

#### **B.6.9.5 Proposed treatment: first aid measures, antidotes, medical treatment (AII 5.9.5)**

##### First aid

If any symptom of acute poisoning occurs, the work must be stopped at once and all exertion prohibited. Contaminated clothing should be removed, exposed skin, eyes, and hair washed. A physician should be consulted at once. The airway should be cleared of secretion, if necessary. Artificial pulmonary ventilation, intubation or tracheotomy may be necessary in case of severe acute poisoning. If indicated, pulmonary ventilation can be provided mechanically as long as respiratory drive is depressed. Complete rest for 24 hours at least, e.g., in hospital, is recommended.

##### Therapeutic regimes

Atropine sulphate is an appropriate antidote, whereas morphine and oximes, e.g., pralidoxime (2-PAM), are contra-indicated. Atropine does not reactivate the cholinesterase enzyme or accelerate excretion or breakdown of methomyl. The objective of atropine treatment is to antagonise the effects of excessive concentrations of acetylcholine at end organs having muscarinic receptors. Atropine is ineffective against nicotinic actions, specifically muscle weakness and respiratory depression. Depending on the severity of poisoning, doses of atropine ranging from small to very large may be required. Tissue oxygenation shall be improved as much as possible before administering atropine to minimise the risk of ventricular fibrillation. Atropine sulphate can be administered intravenously or intramuscularly until atropinisation is adequate. Atropinisation is maintained by repeated doses for 2-12 hours or longer, if necessary, depending on the severity of poisoning. Rales in lung bases, miosis, nausea, bradycardia, and other cholinergic manifestations nearly always indicate inadequate atropinisation. Severely poisoned individuals may exhibit remarkable tolerance to atropine.

#### **B.6.9.6 Expected effects of poisoning (AII 5.9.6)**

The toxic effects of acute poisoning are reversible and decline rapidly. Reduced body weight gain and temporary alterations in clinical chemistry including liver enzymes and glucose levels may be observed. Tests in laboratory animals demonstrate no serious health effects of long term exposure. Methomyl is neither a carcinogen nor

reproductive or developmental toxin in animals, nor is it a genotoxin in *in vitro* and *in vivo* tests.

## **B.6.10 Summary of mammalian metabolism and toxicity, proposed ADI, AOEL and drinking water limit**

### **B.6.10.1 Summary of mammalian toxicity**

Methomyl was readily absorbed from the gastrointestinal tract (only 2-4% eliminated in faeces) and rapidly eliminated within 24 hours of dosing (80% in the rat and 63% in the monkey). Urinary excretion accounted for 53% of the administered dose in rats and 29% in monkeys. Expired air accounted for approximately 33% of the administered dose in rats and 39% in monkeys. The excretion half-life was about 5 hours in the rat and between 12 and 24 hours in the monkey. There were no sex differences in the absorption, the rate of elimination or in the distribution and concentration of the tissue residues in rats. At termination, approximately 8-10% of the dose was retained in the rat tissues and approximately 5% in the monkey. Total radioactive tissue levels in rats were lower or similar to plasma levels, indicating no specific bioaccumulation with the exception of some retention of radioactivity in red blood cells.

Metabolism was extensive in both the rat and monkey but there are some differences in the metabolic pathways and profiles. In the rat, the metabolite profiles in the urine were nearly identical between male and female rats. The major urine metabolite was the mercapturic acid derivative of methomyl (IN-KA129) together with at least 10 other minor urinary metabolites. Acetonitrile was the major residue in blood and liver. In the monkey, over 18 metabolites were observed, none of which were greater than 4% and included those metabolites common with the rat. The monkey excretes more  $^{14}\text{CO}_2$  and less  $^{14}\text{C}$ -acetonitrile than the rat in expired air, the monkey excretes considerably less of the mercapturic acid derivative of methomyl in urine (0.8% in monkey v18% in rat) and the monkey excreted a greater number of urinary metabolites.

Three major pathways were proposed for the rat: displacement of S-methyl from *syn*-methomyl by glutathione followed by transformation to the mercapturic acid derivative; conversion of methomyl to methomyl oxime and  $\text{CO}_2$  release; and isomerisation of *syn*-methomyl to *anti*-methomyl (IN-B1884), followed by a Beckmann rearrangement and formation of acetonitrile (IN-07467). Two major pathways were proposed for the monkey: hydrolysis of the carbamate ester of methomyl to methomyl oxime which is subsequently metabolised to  $\text{CO}_2$ ; and isomerisation of *syn*-methomyl to *anti*-methomyl, followed by a Beckmann rearrangement and formation of acetonitrile. A minor pathway involved displacement of S-methyl from *syn*-methomyl by glutathione followed by transformation to the mercapturic acid derivative (IN-KA129). Acetonitrile was further metabolised via conjugation with cysteine and sulphate.

Methomyl has a high order of acute toxicity in experimental animals via the oral, ocular and inhalation routes of exposure, but it has a relatively low order of acute toxicity via the dermal route. Intentional (suicides) and accidental human exposures indicate that fatalities can occur at oral doses as low as 12 mg/kg bw. Although the

results of the acute oral and inhalation studies are borderline, the weight of evidence indicates that methomyl should be classifiable as Very Toxic (by inhalation) and Toxic (if swallowed) based on the submitted GLP-compliant studies. Additional oral toxicity studies (see footnote to table 6.12) indicate a higher classification might be appropriate. Methomyl is unclassified via the dermal route of exposure. It is not an eye or skin irritant and does not cause skin sensitisation.

Short-term feeding studies have been conducted in rats, mice and dogs (2-year dog study included in this section). They have not been conducted to modern protocols or standards. None of these studies carried out ophthalmological examinations or carried out reliable determinations to evaluate brain cholinesterase activity. Where cholinesterase activities have been determined, the results are considered to be equivocal because of deficiencies in the reporting and/or the methodology. The overall quality of the short-term oral studies is below that normally expected and inadequate for use in the setting of reference values. However, several additional studies were performed to assess the degree and reversibility of cholinesterase inhibition (B.6.8.2-6.8.3).

The most sensitive endpoint in the short-term feeding studies appears to be the haematological changes seen in mice (at 150 ppm) and rats (at 270 ppm). In the 2-year dog study, the most sensitive endpoints appear to be the organ weight and histopathological changes in the kidneys (pigment disposition and slight swelling of the epithelial cells of the proximal convoluted tubules) and spleen (pigment deposition and extramedullary haematopoiesis) at dose levels  $\geq 400$  ppm. Haematological changes and mortality were evident in dogs at 1000 ppm. These feeding studies are considered inappropriate for the setting of reference doses for regulatory purposes on a stand-alone basis.

Two 21-day dermal toxicity studies were conducted in rabbits. In the first study, statistically significant decreases in plasma and brain cholinesterase activities, as well as increased incidences of hyper-reactivity, were observed in male and female rabbits at the high dose (500 mg/kg bw/day). In the second study, there were no clear treatment-related effects on plasma, red blood cell or brain cholinesterase activities. A NOAEL of 90 mg/kg bw/day was determined for this second study, the highest dose used, however some equivocal clinical signs and cholinesterase inhibition patterns were noted, which were difficult to interpret with the small group size of 6 rabbits. In the first study (occlusive applications), the methomyl was moistened with approximately 5 ml of deionised water under an occlusive dressing while in the second study (semi-occlusive applications) the methomyl was moistened by 1 ml of deionised water (test site was 190 cm<sup>2</sup> of shaved skin in both studies).

The company has submitted a battery of genotoxicity studies (study dates 1984-1995) in which the mutagenic and DNA-damaging potential of methomyl has been evaluated in accordance with the protocols of international test guidelines. Negative results were obtained in all of these studies (including an *in vivo* clastogenicity assay and a bone marrow micronucleus assay). Positive results have been reported in the literature but no published studies were submitted for evaluation. The notifier has evaluated two of these published papers and submitted a summary of their findings. Based on deficiencies in the study protocols and the route of administration (i.p. injection) in the *in vivo* study, the company considers these publications to be unsuitable for assessing

the genotoxic potential of methomyl. Methomyl was not oncogenic in long-term studies conducted in rats and mice and did not induce reproductive or developmental toxicity in studies performed in rats and rabbits. Based on the results of the company studies, the weight of the evidence indicates that methomyl does not pose a mutagenic or genotoxic concern.

In the long-term rat study, body weight effects and mild haematological changes were observed (reduced RBC count, haemoglobin and haematocrit). In the long-term mouse study, reduced survival and mild transient haematology changes (as seen in rats) were observed (the haematological changes were not present after 26 weeks). There was no evidence of methomyl-induced carcinogenic activity in rats or mice.

In the two-generation reproduction toxicity study, the main effects on parental animals were reduced body weight and food consumption and increased relative spleen weight. There were no effects on reproduction and fertility but the combined mean pup weights were reduced (male and female data not reported separately). In the three generation reproduction toxicity study, there were no effects on parents or reproduction and fertility. Pup weight and food consumption were reduced in F3 pups.

Developmental toxicity studies were conducted in rats and rabbits. In the rat developmental study, the maternal effects included reduced body weight and food consumption. There were no effects on the rat foetuses. In the rabbit developmental study, deaths, decreased body weight and clinical signs of cholinesterase activity were observed in the dams. There was no evidence of methomyl-induced teratogenic activity in rats or rabbits.

Modern neurotoxicity studies have been conducted in the rat and a delayed neurotoxicity study was conducted in hens in the 1960s. Following an acute oral (gavage) dose of methomyl in water to rats, clinical signs of systemic toxicity and cholinesterase inhibition (plasma, erythrocyte and brain) were observed in both sexes at peak exposure (30 minutes post dosing). It was noted that fore and hind limb grip strength was not affected by treatment. A NOAEL of 0.25 mg/kg bw was determined for reversible dose-related brain cholinesterase activity at the next highest dose. Clinical signs were evident at 1 mg/kg bw in a pilot study (tremors, low posture, abnormal gait and uncoordinated righting reflex). Following dietary administration for 90-days to rats, there were effects on body weight, food consumption, clinical signs of systemic toxicity, brain cholinesterase activity and some of the FOB parameters at the top dose level. Although clinical signs, FOB effects and reduced brain cholinesterase activity were observed, no effects on plasma or erythrocyte cholinesterase activities were detected. After 13 weeks of dosing, male forelimb and hindlimb grip strength was reduced at the top dose level. Although there was an effect on brain cholinesterase activity following dietary administration, there were no effects on plasma or erythrocyte cholinesterase activities. A NOAEL of 150 ppm was determined for both sexes (equivalent to 9.42 and 11.2 mg/kg bw/day, respectively). There was no evidence of delayed toxicity in the hen test.

Several additional studies were performed to assess the reversibility of cholinesterase inhibition in rats, acute oral administration in rats (gavage and dietary bolus dosing), repeated oral dosing in rats (gavage dosing) and the *in vitro* activity of human and rat cholinesterase. A human volunteer study was also carried out to evaluate

cholinesterase activity and the potential clinical signs of systemic toxicity in humans. The results of the *in vivo* studies and the neurotoxicity studies are presented in Table 6.79.

WARNING: This document forms part of an EC evaluation data package and should not be read in isolation. Registration must not be granted on the basis of this document.

Table 6.80. Summary of the cholinesterase data

Study details	NOAEL	LOEL	Reference
Acute oral administration in male volunteers (n = 5/group) (capsule/bolus dosing)	0.1 mg/kg bw	0.2 mg/kg bw: RBC cholinesterase depression ( $\geq 20\%$ ) & increased salivation.  0.3 mg/kg bw: RBC cholinesterase depression ( $\geq 20\%$ ), increased salivation & headache.	McFarlane <i>et al</i> , 1998.
<sup>a</sup> Acute oral administration in male rats (dietary/bolus dosing)	30 ppm (1.0 mg/kg bw)	60 ppm (1.88 mg/kg bw): RBC cholinesterase depression ( $\geq 20\%$ ) & no reaction to tail pinch. 120 ppm (3.74 mg/kg bw): RBC and brain cholinesterase depression ( $\geq 20\%$ ) & no reaction to tail pinch. 360 ppm (9.98 mg/kg bw): plasma, RBC and brain cholinesterase depression ( $\geq 20\%$ ), no reaction to tail pinch and other behavioural effects.	Filliben, 1996
Acute oral (gavage) administration in male and female rats (reversibility study).	Not determined	<sup>b</sup> 3.0 mg/kg bw: Plasma, RBC and brain cholinesterase depression ( $\geq 20\%$ ) & clinical signs.	Malley, 1997
<sup>c</sup> Acute neurotoxicity study in male and female rats (gavage).	0.25 mg/kg bw for males and females	0.5 mg/kg bw: Reversible red blood cell and brain cholinesterase inhibition ( $\geq 20\%$ ).	Mikles, 1998a
Ten day oral (gavage) administration in male rats.	Not determined	5.1 mg/kg bw/day: clinical signs shortly after dosing	Sherman, 1964
90-day (dietary) neurotoxicity study in male and female rats.	150 ppm (9.42 and 11.2 mg/kg bw/day for males and females.	1500 ppm: Reduced body weight and food consumption, clinical signs, brain cholinesterase inhibition and effects on FOB parameters.	Mikles, 1998b

Key: a) Rats preconditioned to eat within a two-hour period. b) Recovery was complete by 3 hours post dosing. c) Clinical signs were evident at 1 mg/kg bw in a pilot study (tremors, low posture, abnormal gait and uncoordinated righting reflex).

In the human volunteer study, there were statistically significant depressions in RBC cholinesterase activity at doses of 0.2 (0.75-1.75 post dosing) and 0.3 mg/kg bw (0.5-1.75 hours post dosing), a single occurrence of a mild headache at a dose of 0.3 mg/kg bw, and quantitatively increased salivation at 0.2 and 0.3 mg/kg bw. Red Blood cholinesterase activity was depressed by 19% at 0.1 mg/kg bw at 1.25 hour post dosing ( $<20\%$  cut off). Four of the subjects in the 0.1 mg/kg bw group had red blood cell cholinesterase depression ( $>20\%$ ). The red blood cell cholinesterase activity in the remaining subject was greater than the baseline for all the post-dosing time point except one. The baseline for this individual was noticeably lower than the other members of the group. Plasma cholinesterase activity was depressed at 0.2 mg/kg bw and above. However, it should be noted that clinical symptoms have been reported in humans at an estimated acute oral dose level of 0.15 mg/kg bw (estimated range 0.09-0.31 mg/kg bw) in an epidemiology study.

The *in vitro* assays comparing human AChE with rat whole blood (AChE and BuChE) cholinesterase showed that the concentration of methomyl required to produce a 50% reduction in human enzyme activity ( $0.265 \times 10^{-5}$  M) was approximately 6-fold lower compared to the rat ( $1.56 \times 10^{-5}$  M).. The regeneration half-life ( $t_{0.5}$ ) was slightly longer for human AChE (38.0 minutes) compared to rat AChE/BuChE (26.6 minutes). However, interpretation of the results is confounded by the fact that the human and rat enzyme samples were not comparable and the use of a limited concentration range.

In residue trials (B.7.6.3), methomyl was found in all trials in the range 0.02-0.59 mg/kg but since methomyl oxime levels were not determined these values are considered to be an underestimation of the total residues. The processing studies (B.7.8.1) indicate that in certain circumstances there may be significant levels of methomyl oxime in processed products. Although this metabolite was not identified in the rat metabolism study and no toxicity data have been submitted on the methomyl oxime itself, based on the metabolism of this metabolite and structural considerations it has been concluded that methomyl oxime was tested in the toxicity studies and does not give rise to toxicological concerns (B.6.8.1). Further reassurance would be required if it was present as a residue at levels significantly higher than the parent.

Table 6.81. The proposed NOELs/NOAELs and LOELs for the standard toxicity studies

Type of study	NOELs/NOAELs	LOEL/effects	Reference
21-day dermal study in rabbits.	50 mg/kg bw/day for male and female	500 mg/kg bw/day: Brain cholinesterase inhibition, haematological changes and clinical signs.	Brock, 1989
21-day dermal study in rabbits.	90 mg/kg bw/day for male and females	90 mg/kg bw/day: the top dose used	Finlay 1997
90-day feeding study in rats.	50 ppm (4.1 & 3.6 mg/kg bw/day in males and females, respectively).	250 ppm: Body weight and food consumption effects. Increased erythroid hyperplasia (males) and reduced RBC parameters (females).	Busey, 1966
90-day feeding study in rats.	Males: 200 ppm (13.6 mg/kg bw/day). Females: 135 ppm (10 mg/kg bw/day).	400 ppm: Haematological changes. 270 ppm: Haematological changes.	Cox, 1979a
35-week feeding study in rats.	100 ppm (only females evaluated) 300 ppm (15 mg/kg bw/day) for both sexes.	300 ppm: Haematological changes.  600 ppm: body weight effects	Cox, 1980
90-day feeding study in mice.	Males: 150 ppm (26.6 mg/kg bw/day). Females: 75 ppm (15.5 mg/kg bw/day).	300 ppm: Haematological changes. 150 ppm: Haematological changes.	Cox, 1979b
23-week feeding study in mice.	100ppm: 100 ppm (16.6 & 23.2 mg/kg bw/day, respectively).	300 ppm: Haematological changes.	Cox, 1979
90-day feeding study in dogs.	400 ppm: (14.7 & 12.5 mg/kg bw/day in males and females, respectively).	400 ppm: top dose used.	Sherman, 1967
2-year feeding	100 ppm (3.0 mg/kg	400 ppm: organ weight and	Busey, 1968



study in dogs	bw/day for both sexes)	histopathology changes in the kidney and spleen.	
2-year chronic /carcinogenicity study in rats	100 ppm (4.8 & 6.3 mg/kg bw/day in males and females, respectively)	400 ppm: Body weight effects and haematological changes.	Kaplan, 1981
2-year chronic /carcinogenicity study in mice	Males: Not determined Females: 50 ppm	50 ppm: Reduced survival. 75 ppm: reduced survival.	Snyder, 1981

Table 6.81 (continued)

Type of study	NOELs/NOAELs	LOEL/effects	Reference
Multigeneration study in rats (two generations).	Parental: 75 ppm (4.6 and 6.7 mg/kg bw/day for F0 males and F1 males, respectively & 4.8 and 6.3 for F0 females and F1 females, respectively).  Reproduction: 1200 ppm.  Pup growth and development: 75 ppm	600 ppm: reduced body weight and food consumption.  Top dose used.  600 ppm: Reduced pup weight.	Lu, 1983
Multigeneration study in rats (three generations).	Parental: 100 ppm (8 mg/kg bw/day).  Reproduction: 100 ppm (8 mg/kg bw/day).  Pup growth and development: 50 ppm (4 mg/kg bw/day).	Top dose used.  Top dose used.  100 ppm: pup weight effects.	Busey, 1968
Developmental study in rats.	Maternal: 100 ppm (9.4 mg/kg bw/day).  Pup development: 400 ppm (339 mg/kg bw/day).	400 ppm: Reduced body weight.  Top dose used.	Culik & Rogers, 1978.
Developmental study in rabbits.	Maternal: 6 mg/kg bw/day.  Pup development: 16 mg/kg bw/day.	16 mg/kg bw/day: Deaths, decreased body weight and clinical signs of cholinesterase activity.  Top dose used.	Feussner <i>et al</i> , 1983.

#### B.6.10.2 Acceptable daily intake (ADI)

The most sensitive toxicological effect of methomyl was a rapid but reversible inhibition of cholinesterase following bolus dosing (i.e. acute gavage and dietary studies and capsular study in human volunteers). In the standard repeat-dose feeding studies, there were reductions in body weight and food consumption, mild regenerative (reversible) haematological changes, increased kidney and spleen weights and histological changes in the kidneys (pigment disposition and slight swelling of the epithelial cells of the proximal convoluted tubules) and spleen (pigment deposition and extramedullary haematopoiesis). In the 90-day neurotoxicity study, clinical signs, brain cholinesterase inhibition and effects on FOB parameters were observed. Methomyl was not carcinogenic, genotoxic or a reproductive toxin.

Normally, the results obtained from the longer-term (subchronic and chronic) studies are used for the calculation of an acceptable daily intake (ADI). Hence, the company has proposed an ADI derived from the NOAEL of 3 mg/kg bw/day determined for the 2-year dog study. However, there are clear effects on cholinesterase activity following

single oral bolus doses in rats (gavage and dietary) and human volunteers (capsules) at dose levels lower than 3 mg/kg bw/day (Table 6.80). In addition, clinical symptoms have been reported in humans at an estimated acute oral dose level of 0.15 mg/kg bw (estimated range 0.09-0.31 mg/kg bw) in an epidemiology study. These data are relevant to the consumer risk assessment and the lowest NOAEL determined for the most sensitive species (i.e. humans) must be used to set the reference doses for consumers.

Based on the NOAEL of 0.1 mg/kg bw determined for male volunteers and an assessment factor of 10 x 2 (10 for intra species variation and 2 for the small group size, wide inter-individual variations in the study and clinical symptoms reported in humans at the estimated LOEL of 0.09 – 0.31 mg/kg bw), **an ADI of 0.005 mg/kg bw/day can be proposed.**

This proposed ADI is supported by the alternative ADI based on animal data; i.e. 0.0025 mg/kg bw/day based on the NOAEL of 0.25 mg/kg bw/day determined for the acute rat study (Mikles, 1998a) and an assessment factor of 100).

#### Company's proposal for the ADI

*The ADI pertains to the amount of a substance that can be consumed safely through the diet over the course of the lifetime of an individual. For methomyl, the lowest NOAEL, 3 mg/kg bw/day, was seen in the 2-year dog study and was based on histopathology changes in the kidney and spleen at higher doses. Using a safety factor of 100 (10X intraspecies variability factor and 10X interspecies extrapolation factor), the ADI is 0.03 mg/kg bw/day.*

#### **B.6.10.3 Acute reference dose (ARfD)**

An ARfD is considered necessary for methomyl based on its mechanism of action and its acute oral toxicity profile. The acute reference dose is intended to represent a limit value of a pesticide that may be consumed as a result of eating at a single sitting. In setting the ARfD, both the acute and short-term studies (including developmental studies) are taken into consideration.

There are no specific reproductive effects. The acute data reviewed indicates that humans are the most sensitive species to methomyl-induced cholinesterase inhibition.

Based on the NOAEL of 0.1 mg/kg bw determined for male volunteers and an assessment factor of 10 x 2 (10 for intra species variation and 2 for the small group size, wide inter-individual variations in the study and clinical symptoms reported in humans at the estimated LOEL of 0.09 – 0.31 mg/kg bw), **an ARfD of 0.005 mg/kg bw/day can be proposed.**

#### Company's proposal for the ARfD

*The ARfD is an estimate of the amount of a substance in food that can be ingested over a short period of time, usually during one meal or one day, without appreciable health risk to the consumer. For methomyl, the proposed ARfD is established on the basis of the no-observed-effect level (NOAEL) of 0.1 mg/kg bw obtained in the acute oral study*

*in human volunteers. The NOAEL was based on increased saliva secretion and decreased RBC and plasma cholinesterase activities at 0.2 mg/kg bw and represents the lowest NOAEL observed in studies relevant to the acute dietary exposure of humans to methomyl. Using a safety factor of 10 (intraspecies variability factor), the ARfD is 0.01 mg/kg bw.*

#### B.6.10.4. Acceptable operator exposure level (AOEL)

The company has not proposed a systemic AOEL for methyl but they have submitted a case for the use of a dermal AOEL and an inhalation AOEL for the operator exposure risk assessment.

##### a) Systemic AOEL

In view of the short-term exposure of operators to methomyl, it is generally considered appropriate to use no observed adverse effect levels from relevant subchronic oral studies in setting a systemic AOEL. In addition, the reproductive effects observed in the developmental studies are also taken into consideration. However, the data reviewed indicated that humans were the most sensitive species to methomyl-induced cholinesterase inhibition; therefore, the NOAEL determined in the single dose human volunteer study is considered to be relevant to the setting of a systemic AOEL.

Based on the NOAEL of 0.1 mg/kg bw determined for male volunteers and an assessment factor of  $10 \times 2$  (10 for intra species variation and 2 for the small group size, wide inter-individual variations in the study and clinical symptoms reported in humans at the estimated LOEL of 0.09 – 0.31 mg/kg bw), **a systemic AOEL of 0.005 mg/kg bw/day can be proposed (adjustment for gastrointestinal absorption is not necessary).**

##### b) Dermal AOEL

Two rabbit dermal studies have been submitted for evaluation. The studies used small groups of rabbits for which there are no comparable oral data and some equivocal findings were seen. Both of these studies were performed using methomyl moistened with deionised water (5 ml or 1 ml) and evaluated cholinesterase activity (critical endpoint). However, operators will be exposed to the formulation (a soluble concentrate) which contains high levels of solvents ( [REDACTED] ). Hence, the material tested in the dermal studies is not representative of the material to which the operators will be exposed (i.e. the absorption characteristics of methomyl from the formulated product might be significantly different from the material tested). Although dermal absorption studies have been performed using the formulation product, cholinesterase inhibition has not been evaluated in these studies. Furthermore, there are no dermal metabolism data or information on the pattern of use. Therefore, it is considered to be inappropriate to set a dermal AOEL at the present time on the dermal data submitted.

*A summary of the company's reasoned case and proposals for setting a dermal AOEL  
(edited by rapporteur)*

*For pesticide operators, there are two primary routes of exposure, dermal and inhalation. Based on the toxicology studies presented in this dossier, it is apparent that there is a fundamental difference in the toxicity of methomyl by the dermal route relative to both the inhalation and oral routes. As noted in preceding sections, methomyl's effects are primarily acute in nature and are related to the inhibition of cholinesterase which occurs quickly following either oral or inhalation exposure (within ~30-60 minutes of dosing) and reverses rapidly (within 2 hours of dosing). However, the dermal toxicity of this substance is comparatively low.*

*Methomyl's low dermal toxicity is attributed to the low rate and extent of dermal penetration coupled with rapid metabolism and elimination and rapid reversibility of cholinesterase inhibition. The combination of these factors subsequently results in low blood concentrations of the toxicologically active molecule (methomyl), and a corresponding low degree of toxicity.*

*The low dermal absorption of methomyl has been demonstrated with a 20% liquid ethanol-dibasic ester based formulation (study summarised in section B.6.12). In this study, the absorption of <sup>14</sup>C-methomyl through the skin of rats was measured following topical application of both the undiluted concentrate (~84 mg/kg bw) and a 133-fold aqueous dilution of the concentrate (0.61 mg/kg bw), doses which were designed to mimic potential field-use exposures. The systemically-absorbed portion of the dose was similar at all sampling times and averaged 5.32% for the undiluted concentrate and 15.56% for the aqueous dilution.*

*Taking into consideration the suggested guidance for establishing AOELs provided by the EU Commission (EU Draft Guidance for the Setting of an Acceptable Operator Exposure Level (AOEL), 7531/VI/95 rev.; 03/01/2001), separate dermal and inhalation AOELs are proposed for methomyl. This approach is considered to be the most appropriate method for assessing potential risk to operators since the degree of toxicity produced by methomyl is route-dependent and can be attributed to fundamental differences in the rate and extent of absorption.*

*Based on the NOAEL of 90 mg/kg bw/day determined for the 21-day dermal study in rabbits (Finlay, 1997) and a standard assessment factor of 100, a dermal AOEL of 0.9 mg/kg bw/day can be proposed. This study is an appropriate study for establishing a dermal AOEL for two reasons: 1) the study was conducted by the route of interest, and 2) the most critical and sensitive toxicological endpoint for methomyl-induced toxicity (cholinesterase inhibition) was assessed. In lieu of similar route-specific data in humans, the 21-day dermal study in rabbits represents a scientifically valid approach for assessing the potential risk to operators by dermal exposure. In addition, an assessment of the dermal penetration of methomyl through rat, rabbit, and human skin in vitro has demonstrated that penetration is greatest through rat and rabbit skin relative to human skin. Therefore, the rabbit is a conservative model upon which to base the dermal AOEL.*

## c) Inhalation AOEL

No repeat dose inhalation studies have been submitted for evaluation.

Two acute inhalation studies have been submitted for evaluation (one using methomyl and one using the formulated product). In the study with methomyl, deaths occurred at 0.182/0.179 mg/l (gravimetric/analysed) and above. Prior to death these decedents exhibited clinical signs typical of reduced cholinesterase activity. Gross necropsy revealed expanded lungs and oedema of the pleural cavities. In the study with the formulation, deaths and clinical signs typical of reduced cholinesterase activity occurred at 0.88 mg/l and above. The mean lung weight to body weight ratios were elevated in the decedents and two rats had congestion of the lungs. Microscopy revealed minimal dilation of renal convoluted tubules in the decedents. NOAELs were not established for cholinesterase activity in these acute studies and the lung effects appear to be dependent on the route of exposure. Therefore, it is considered to be inappropriate to set an inhalation AOEL at the present time.

Company's proposed inhalation AOEL

*The inhalation AOEL is most appropriately based on an inhalation study in which cholinesterase inhibition was assessed. However, in lieu of such data for methomyl in humans or a clear NOAEL in laboratory animals, the use of an acute oral study is proposed as a surrogate for the inhalation route of exposure. The proposed inhalation AOEL, therefore, is established on the basis of the NOAEL of 0.1 mg/kg bw obtained in the acute oral study in human volunteers.*

*Based on the NOAEL of 0.1 mg/kg bw determined for the human volunteer study and an assessment factor 10 (intraspecies variability factor), an inhalation AOEL of 0.01 mg/kg bw can be proposed (adjustment for gastrointestinal absorption is unnecessary).*

#### B.6.10.5 Maximum admissible concentration in drinking water (MAC value)

Using the WHO 1994 model to calculate the MAC for drinking water it is appropriate to divide the ADI by an additional assessment factor of 10 and thus derive an intake of 0.0005 mg/kg bw/day.

Assuming the average value for consumption by a typical 60 kg person is 2 litres/day, a daily intake of 0.0005 mg/kg bw/day would be achieved by drinking water containing 0.015 mg/litre. Thus, **a MAC of 15 µg/l of water can be derived for methomyl.**

Company's proposal for the MAC

*On the basis that exposure through the drinking water should not account for more than 10% of the ADI, assuming average consumption of 2 litres of water per person per day and a body weight of 60 kg, a limit of 0.09 mg/l is proposed for methomyl (see calculation below).*

$$\frac{0.03 \text{ mg/kg bw/day (ADI)} \times 0.1 \text{ (represents 10\% factor)} \times 60 \text{ kg (weight of avg. person)}}{2 \text{ litres water/day}}$$

**B.6.11 Acute toxicity, irritancy and skin sensitisation of the preparation**

Methomyl 20 L (synonym: Methomyl 20 SL) is a soluble concentrate containing 200 g/l methomyl.

**B.6.11.1 Acute oral toxicity in rats (AIII 7.1.1)**

Study type	Acute oral toxicity		
Reference	DuPont-3282	GLP Certified:	Yes
Formulation:	Methomyl 20L	Guideline:	OECD 401 (1987) ≅ 92/69/EEC B.1
Appearance	Blue liquid		
Test System:	Rat: CrI:CD (SD) IGS BR (5/sex/dose).	Year(s) of Conduct:	1999
Doses:	15, 30, 75 or 150 mg/kg bw (gavage).	Vehicle:	Deionised water.
Observation period:	14 days	Application volume:	10 ml/kg bw.

The animals were fasted for 16-17 hours prior to dosing. Individual dose volumes were calculated using the fasted body weights obtained prior to dosing.

**Results**

Dose level (mg/kg bw)	Deaths	
	Males	Females
15	0/5	0/5
30	0/5	0/5
75	0/5	0/5
150	3/5 (day 1)	5/5 (day 1)

Clinical signs of toxicity were observed in males at 75 mg/kg bw and above and in females at 30 and 75 mg/kg bw. These signs included salivation, staining of various body parts, tremors, fasciculations, wet chin and alopecia. Apart from alopecia and staining, all the clinical signs were observed on the day of dosing. There were no body weight effects and no gross abnormalities at necropsy. The combined acute oral LD<sub>50</sub> for male and female rats was estimated to be 132 mg/kg bw. Therefore, the test substance is classifiable as Toxic (if swallowed) via the oral route according to EC criteria.

(Finlay, 1999)

**B.6.11.2 Acute dermal toxicity (AIII 7.1.2)**

Study type	Acute dermal toxicity (limit test)		
Reference	DuPont-3193	GLP Certified:	Yes
Formulation:	Methomyl 20L	Guideline:	OECD 402 (1987) ≅ 92/69/EEC B.3
Appearance	Blue liquid		
Test System:	Rat: CrI:CD (SD) IGS BR (5/sex/dose).	Year(s) of Conduct:	1999
Doses:	5000 mg/kg bw.	Vehicle:	Distilled water
Area covered	37 cm <sup>2</sup> , clipped scapular to lumbar parts of the trunk (occluded dressing).	Application volume:	1-1.5 ml/kg bw
Duration of exposure	24 hours, then washed clean with soap and warm water.	Observation period:	14 days

Skin reactions were scored on the Draize scale.

## Results

No deaths occurred during the study. Slight erythema was observed in 2 rats on the day after application but all skin reactions had resolved within six days of treatment. Seven rats had a black ocular discharge and 3 had a black nasal discharge on the day after treatment. A wet and yellow stained perineum was observed in one rat on the day of treatment. Weight loss of approximately 5-10% of the initial body weight was observed in 9 rats on the day of after treatment. The report believed these clinical signs were due to stress induced by the application of the occluded dressings. No gross findings were seen at necropsy. The dermal LD50 value was greater than 5000 mg/kg bw for both sexes. Therefore, the test substance is not classifiable via the dermal route according to EC criteria.

(Finlay, 1999a)

### B.6.11.3 Acute inhalation toxicity (AIII 7.1.3)

Study type	Acute inhalation (limit test)		
Reference:	DuPont DPT 247/91521	GLP Certified:	Yes
Formulation:	Methomyl 20L	Guideline:	OECD 403 $\cong$ 92/691/EEC B.2
Appearance	Blue liquid		
Test System:	Rat: Sprague Dawley (5/sex/dose).	Year(s) of Conduct:	1991
Doses:	0.30, 0.88, 1.12 or 1.86 mg/l	Vehicle:	None
Test material	A liquid droplet aerosol	Exposure:	Nose-only (4 hours).
		Observation period:	14 days

## Results

### Exposure conditions

Nominal concentrations	Analysed concentrations	Particle size (MMAD/GSD) <sup>a</sup>	Particles < 6 $\mu$ m (% w/w)	Chamber temperature
0.93 mg/l	0.30 mg/l	2.3 $\mu$ m/2.14	90%	24 °C
4.29 mg/l	1.86 mg/l	2.9 $\mu$ m/2.18	84%	25 °C
2.78 mg/l	0.88 mg/l	2.7 $\mu$ m/2.25	83%	24 °C
3.28 mg/l	1.12 mg/l	2.1 $\mu$ m/2.17	92%	24 °C

Key: a) mass median aerodynamic diameter (MMAD) and geometrical standard deviations (GSD). b) Chamber humidity was not recorded. c) Air flow 4 litres/minute (the report did not indicate that the chamber airflow was recorded or monitored continuously).



Mortality

Dose level (mg/l)	Deaths	
	Males	Females
0	0/5	0/5
0.30	0/5	0/5
0.88	0/5	1/5 (within 10 minutes of the end of exposure)
1.12	3/5 (2 during exposure 1 after 1 hour the end of exposure)	2/5 (2 during exposure)
1.86	4/5 (3 during exposure 1 within 5 minutes of the end of exposure)	4/5 (3 during exposure 1 within 5 minutes of the end of exposure)

Clinical signs during exposure included wetness around the mouth, ataxia, irregular respiration and fascicular tremors at 0.88 mg/l and above. During the observation period, tremors, gasping and respiratory abnormalities, exophthalmus and lethargy were observed at 0.88 mg/l and above. All the surviving animals appeared normal by day 7. There were moderate to marked decreases in bodyweight or reductions in body weight gain for up to 3 days after exposure. Subsequent weight gain for surviving rats was similar to controls. Reduced food consumption (marked at the higher exposure levels) was noted for up to six days following exposure. Water consumption was reduced for up to 5 days following exposure at 0.88 mg/l and above.

Mean lung weight to body weight ratios (lung weight x 100/bw)

Dose (mg/l)	Survivors		Decedents	
	Males	Females	Males	Females
0	0.47 (0.056) <sup>a</sup>	0.53 (0.025)	-	-
0.30	0.45 (0.020)	0.58 (0.021)	-	-
0.88	0.47 (0.014)	0.58 (0.026) [n=4]	-	0.81 [n=1]
1.12	0.53 [n=2]	0.50 (0.017) [n=3]	0.72 (0.087) [n=3]	0.69 [n=2]
1.86	0.53 [n=1]	0.49 [n=1]	0.64 (0.101) [n=4]	0.62 (0.061) [n=4]

Key: a) Standard deviation.

The mean lung to body weight ratios were elevated in the decedents as compared to the control values. Two rats that died had congestion of the lungs. Most other rats had no abnormality except stained fur.

Microscopic findings-the incidence of minimal dilation of the renal convoluted tubules

Dose (mg/l)	Survivors		Decedents	
	Males	Females	Males	Females
0	0/5	0/5	-	-
0.30	0/5	0/5	-	-
0.88	0/5	0/4	-	0/1
1.12	0/2	0/3	3/3	1/2
1.86	0/1	0/1	2/4	1/4

Minimal dilation of renal convoluted tubules was observed at 1.12 mg/l and above in the decedents.

The LC50 values were calculated to be 1.30 and 1.24 mg/l for males and females, respectively. The combined LC50 for both males and females was calculated to be 1.28 mg/l. Therefore, the test substance is classifiable as Harmful (by inhalation) via the inhalation route according to EC criteria.

(Jackson *et al*, 1991)

#### B.6.11.4 Skin irritancy (AIII 7.1.4)

Study type	Acute dermal irritancy		
Reference:	DuPont-3158	GLP Certified:	Yes
Formulation:	Methomyl 20L	Guideline:	OECD 404 (1992) $\cong$ 92/69/EEC B.4
Appearance	Blue liquid		
Test System:	Rabbit: New Zealand White (6 females).	Year(s) of Conduct:	1999
Doses:	0.5 ml	Vehicle:	None
Area Covered:	ca.6 cm <sup>2</sup> , shaved skin from scapular to lumber region (semi-occlusive dressing).	Duration of exposure:	4 hours: then wiped clean with soap and warm water

Skin responses were scored on the Draize scale at 24, 48 and 72 hours after treatment.

#### Results

No clinical signs of toxicity were observed in any of the animals. Although the test substance stained the skin at the application sites, the test sites could be evaluated for erythema. No dermal irritation was detected in any of the animals. Therefore, the test substance is not classifiable as a skin irritant according to EC criteria.

(Finlay, 1999b)

**B.6.11.5 Eye irritancy (AIII 7.1.5)**

Study type	Acute eye irritancy		
Reference:	DuPont-3286	GLP Certified:	Yes
Formulation:	Methomyl 20L	Guideline:	OECD 405 (1987) ≡ 92/69/EEC B.5
Appearance	Blue liquid		
Test System:	Rabbit: New Zealand White (6 animals).	Year Conducted	1999
Dose:	0.1 ml	Vehicle:	None
Applied to:	Instilled into conjunctival sac of right eye	Response recording (Draize scale)	1, 24, 48, and 72 hours, 7, 14 and 21 days.

**Results**

Time	Corneal opacity						Iridial reactions						Conjunctiva											
													Redness						Chemosis					
No	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
1 h	0	2	2	2	2	2	1	1	1	1	1	1	2	3	3	3	3	3	4	4	4	4	4	4
24 h	0	2	2	2	2	2	1	1	1	1	1	1	3	3	3	3	3	3	2	3	3	3	3	3
48 h	0	2	2	2	2	2	0	1	1	1	1	1	3	3	3	3	3	3	1	3	3	3	2	2
72 h	0	2	3	2	1	0	0	1	1	1	1	1	3	3	3	3	3	2	1	2	3	3	2	2
7 d	0	0	0	4	0	0	0	0	0	1	0	0	1	2	1	3	2	2	0	1	0	3	0	1
14 d	0	0	0	2	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
21	-	-	-	2	-	-	-	-	-	0	-	-	-	-	-	0	-	-	-	-	-	0	-	-
Mean	1.56						0.89						2.94						2.44					

Key: a) Mean = average means 24-72 hours.

All rabbits exhibited pupillary constriction, salivation and incoordination within 15 minutes of treatment. Lung noise was noted in 1 rabbit at 1 hour after treatment. The pupils were still constricted at 4 hours after instillation. All of these clinical signs subsided by 24 hours after treatment (dose level approximately 5 mg/kg bw).

Corneal opacity, iritis and conjunctival effects were seen in all the rabbits. Epithelial sloughing of the cornea was seen in four rabbits and corneal vascularization was seen in one rabbit. The mean scores over the 24-72 hour observation period indicate the test substance is a severe eye irritant. In addition, corneal vascularisation was still present in one animal at 21 days post treatment. Therefore, the test substance is classifiable as an eye irritant with the risk phrase 'Risk of serious damage to the eyes' (R41) according to EC criteria.

(Finlay, 1999c)

**B.6.11.6 Skin sensitisation (AIII 7.1.6)**

Study type	Skin sensitisation (Modified Buehler-3 inductions)		
Reference:	DuPont-3022	GLP Certified:	Yes
Formulation:	Methomyl 20L	Guideline	OECD 406 (1992) $\cong$ 92/69/EEC B.6
Appearance	Blue liquid		
Test System:	Guinea Pig: Hartley	Year of Conduct:	
Group Size:	20 test animals & 10 negative control animals.	Positive control:	None (stock tested at regular intervals/data provided).
Topical induction applications		Topical challenge applications	
Left flank	Undiluted test substance	Right flank	Undiluted test substance

**Materials and methods**

Skin reactions were assessed using the following scale: 0 = no redness, 0.5 (+) = very faint redness (usually nonconfluent), 1 = faint redness (usually confluent), 2 = moderate redness, 3 = severe redness with or without swelling.

Dose selection for the induction and challenge phases were based on the results of preliminary studies. Since the undiluted test substance (0.5 ml) did not induce any dermal irritation at 24 or 48 hours, the undiluted test substance was used for the induction and challenge phases.

In the induction phase of the main study, the test material (0.5 ml) was applied to clipped left flank skin under an occlusive protective device/dressing for 6 hours. The protective dressing was removed and the test sites were wiped clean with saline solution and water. The negative control animals received identical treatment apart from the application of the test substance. Approximately 24 and 48 after application, the test sites were scored for skin reactions. This induction procedure was carried out at 7-day intervals for three consecutive weeks.

Fifteen days after the last induction application the test and control animals were challenged on the right flank under an occlusive protective device/dressing for 6 hours. The test sites were scored for skin reactions approximately 24 and 48 after the challenge applications.

**Results**

No skin reactions were observed in the test or negative control animals after the induction and challenge applications. No compound-related clinical signs or body weight effects were noted. Therefore, the test substance was not classifiable as a skin sensitizer according to EC criteria.

(Hershman, 1999)

**B.6.11.7 Summary of the toxicity of the preparation**

Methomyl 20L is moderately toxic via the oral and inhalation routes and of low toxicity via the dermal route. It is not a skin irritant or a skin sensitizer. Methomyl 20L is a severe eye irritant and the presence of corneal vascularisation at 21 days post-instillation warrants the risk phrase R41 (Risk of serious damage to eyes).

Table 6.82. Summary of the acute toxicity, irritancy and skin sensitisation of Methomyl 20L

Test	Species	Result	Comments/ classification	Reference
Acute oral	Rat	LD 50 value: 132 mg/kg bw (combined value for both sexes).	Classified: Toxic if swallowed	Finlay, 1999.
Acute dermal	Rat	LD 50 value: >5000 mg/kg bw for both sexes.	Unclassified	Finlay, 1999a.
Acute inhalation	Rat	LC value: 1.30 mg/l for males and approx. 1.24 for females.	Classified: Harmful by inhalation	Jackson <i>et al</i> , 1991.
Skin irritancy	Rabbit	Non-irritant	Unclassified	Finlay, 1999b.
Eye irritancy	Rabbit	Severe irritation	Classified: Risk of serious damage to eyes (R41).	Finlay 1999c.
Skin sensitisation	Guinea pig	Negative in a Buehler test	Unclassified	Hershman, 1999.

## B.6.12 Dermal absorption

### B.6.12.1 *In vitro*

<b>Report</b>	Fasano, W.J. (2001a). Methomyl: <i>in vitro</i> dermal kinetics of [ <sup>14</sup> C]methomyl in rat, human, and rabbit skin (Lannate 20L formulation). Unpublished DuPont Report No.: DuPont-5835 (in-life phase: 2001).
<b>Test facility</b>	DuPont Haskell Laboratory, P.O. Box 50, Elkton Road, Newark, Delaware, 19714 USA
<b>Guidelines</b>	OECD Guidelines for the Testing of Chemicals (Draft Guideline 428), Skin Absorption: <i>In Vitro</i> Method (December 2000).
<b>Deviations from guidelines</b>	Not applicable
<b>GLP</b>	Yes
<b>Non labelled methomyl</b>	DPX-X1179-512, H22577, chemical purity 98.02%
<b>Radiolabelled methomyl</b>	HOTC 555, H-22705-46, specific activity 42.96 µCi/mg, radiochemical purity >99%.
<b>Formulation blank</b>	H-24819
<b>Acceptable</b>	Yes

## Materials and methods

The dermal penetration and absorption of <sup>14</sup>C-methomyl was measured *in vitro* through rat, human, and rabbit skin. Methomyl 20SL (a formulated preparation containing 20% methomyl.), was applied as a 1.5 g methomyl/l aqueous dilution and as the undiluted concentrate at 200 g methomyl/l. Penetration and absorption were followed using <sup>14</sup>C-methomyl, which was uniformly blended into the Methomyl 20SL formulation prior to application. The amount of active substance applied per area of skin was nominally 15 µg methomyl/cm<sup>2</sup> and 2000 µg methomyl/cm<sup>2</sup> for the 1.5 g/l aqueous dilution and the 200 g/l undiluted concentrate, respectively. The formulation concentrations and application rates were designed to mimic potential field-use

exposures. All measures of radioactivity were expressed as methomyl equivalents or as a percentage of the applied dose.

Table 6.83. Experimental design

Species/group	Dose solution concentration (g/l)	Skin dose level ( $\mu\text{g}$ methomyl/ $\text{cm}^2$ )	<sup>a</sup> No of skin preparations	Target ( $\mu\text{Ci/skin}$ )
Rat	A 1.5	15	12	0.4
	B 200	2000	12	1.0
Human	C 1.5	15	12	0.4
	D 200	2000	12	1.0
Rabbit	E 1.5	15	12	0.4
	F 200	2000	12	1.0

Key: a) Four skin preparations at 3 post-exposure collection points of 0, 6 and 18 hours.

Epidermal membranes were obtained from dorsal skin of female Sprague Dawley rats and human thigh skin (male and female donors aged 50-71). Full-thickness skin was used from the dorsal-flank region of male New Zealand rabbits. The integrity of each preparation was assessed by measuring the electrical resistance prior to application of the test substance. Membranes with a resistance of  $\geq 5.8$  k $\Omega$  (rat),  $\geq 17$  k $\Omega$  (human) and of  $\geq 5.7$  k $\Omega$  (rabbit) were considered intact.

Static diffusion cells with an exposure area of  $0.64$   $\text{cm}^2$  were used. The receptor fluid was 0.9% saline (solubility in water =  $55$   $\text{mg/ml}$  at  $25^\circ\text{C}$ ). The test substance was applied to the skin surface at a rate of  $10$   $\mu\text{l/cm}^2$  for an exposure period of six hours (unoccluded exposure). Samples of receptor fluid were taken from the receptor chambers at 0.5, 1, 2, 4 and 6 hours during the exposure period. A final receptor fluid sample was collected prior to termination for the 6- and 18-hour groups. The volume of the receptor fluid was maintained by the replacement with fresh receptor fluid. At completion of the exposure period, the skin surface was washed, and depletion of skin residues was monitored at 0, 6 and 18 hours post-exposure.

## Results

The analytical data for the dosing solutions are presented in Table 6.84.

Table 6.84. Summary of the formulation analysis and application rates

Test substance	Target concentration (g/l)	Verified (g/l)	Specific activity ( $\mu\text{Ci/mg}$ )	Mean dose ( $\mu\text{Ci}$ )	Amount applied ( $\mu\text{g}$ )	Amount/area ( $\mu\text{g/cm}^2$ )
1.5 g/l aqueous dilution	1.50	1.54	40.8	0.40	9.86	15.4
200 g/l undiluted concentrate	200	192.9	0.77	0.85	1105.0	1726.6

Following topical exposure to the 1.5 g/l aqueous dilution, cumulative penetration of radioactivity was greatest for rat epidermis compared to human epidermis and rabbit skin. A similar observation was made following topical exposure to the 200 g/l undiluted concentrate.

Table 6.85. Cumulative penetration of 14C-methomyl from the 1.5 g/l aqueous dilution

Time (hours)	Mean cumulative penetration ( $\mu\text{g equiv}/\text{cm}^2$ )		
	Rat epidermis	Human epidermis	Rabbit skin
0.5	1.20 (0.42)	NA (NA)	NA (NA)
1	2.53 (0.62)	0.10 (0.08)	NA (NA)
2	3.80 (0.38)	0.30 (0.21)	0.09 (0.01)
4	5.06 (0.33)	0.70 (0.36)	0.32 (0.17)
6 <sup>a</sup> (end exposure)	5.81 (0.15)	1.05 (0.42)	0.67 (0.23)
12 <sup>b</sup> (6 hours post-exposure)	6.77 (0.65)	2.42 (0.93)	1.66 (0.03)
24 <sup>c</sup> (18 hours post-exposure)	6.98 (NA)	3.70 (NA)	2.38 (NA)
<sup>a</sup> Penetration rate 0-6 hrs ( $\mu\text{g equiv}/\text{cm}^2/\text{hr}$ )	0.78	0.19	0.17
<sup>b</sup> Penetration rate 6-12 hrs ( $\mu\text{g equiv}/\text{cm}^2/\text{hr}$ )	0.16	0.21	0.15
<sup>c</sup> Penetration rate 12-24 hrs ( $\mu\text{g equiv}/\text{cm}^2/\text{hr}$ )	0.06	0.05	0.08

Key: a) Mean for all exposures over 0-6 hours. b) Mean data for the 6 and 18 post-exposure groups. c) Mean data for the 18 hour post exposure group.

During the initial 6-hour exposure period, penetration of 14C-methomyl from the 1.5 g/l aqueous dilution was 4-fold faster through rat epidermis ( $0.78 \mu\text{g equiv}/\text{cm}^2/\text{hr}$ ) than through human epidermis ( $0.19 \mu\text{g equiv}/\text{cm}^2/\text{hr}$ ) and rabbit skin ( $0.17 \mu\text{g equiv}/\text{cm}^2/\text{hr}$ ). During the 12-24 hour exposure period, the penetration rates were low for all the preparations.

The recovery data for the aqueous dilution are presented in Table 6.86. The total mean absorption was 52.8% (48.5-57.1%) for rat epidermis, 29.7% (22.3-39.3%) for human epidermis and 43.6% (43.1-44.0%) for rabbit skin. Total recovery ranged from 83-97.7% for the rat preparations, 93.5-98.0% for the human preparations and 95.4-97.0% for the rabbit preparations. There was a decrease in the radioactivity levels in the epidermal and skin preparations at 6 and 18 hours post exposure and a corresponding increase in the levels in the receptor fluid.

Table 6.86. Summary of the recovery data for the 1.5 g/l aqueous dilution

Parameter	Percent of the applied dose)		
	(0 hour post-exposure)	(6 hour post exposure)	(18 hour post-exposure)
Rat epidermis			
Absorbed dose:			
Receptor fluid	37.7 (11.5)	46.9 (11.3)	45.3 (1.61)
Epidermis	15.0 (6.57)	10.2 (5.43)	3.22 (2.05)
Total absorbed	52.7 (17.5)	57.1 (10.8)	48.5 (2.76)
Unabsorbed:			
Epidermal wash	29.2 (7.68)	37.4 (12.4)	49.0 (4.41)
Donor chamber	1.17 (0.69)	0.64 (0.68)	0.08 (0.09)
Total unabsorbed	30.3 (7.49)	38.1 (11.8)	49.1 (4.37)
<b>Total recovered</b>	<b>83.0 (19.2)</b>	<b>95.2 (2.37)</b>	<b>97.7 (1.69)</b>
Human epidermis			
Absorbed dose:			
Receptor fluid	5.45 (2.06)	11.4 (4.18)	24 (4.92)
Epidermis	16.8 (2.13)	16.0 (6.13)	15.3 (2.61)
Total absorbed	22.3 (3.56)	27.4 (10.)	39.3 (2.98)
Unabsorbed:			
Epidermal wash	68.9 (12.2)	70.0 (10.5)	57.2 (4.41)
Donor chamber	2.37 (2.33)	0.55 (0.29)	0.79 (0.40)
Total unabsorbed	71.2 (11.1)	70.6 (10.2)	58.0 (93.57)
<b>Total recovered</b>	<b>93.5 (8.53)</b>	<b>98.0 (1.12)</b>	<b>97.3 (1.68)</b>
Rabbit epidermis			
Absorbed dose:			
Receptor fluid	2.65 (1.17)	10 (5.71)	15.4 (3.13)
Skin	40.4 (3.18)	32.9 (12.0)	28.5 (6.54)
Total absorbed	43.1 (3.03)	43.6 (10.7)	44.0 (592)
Unabsorbed:			
Skin wash	49.4 (2.49)	53.1 (9.99)	52.8 (5.69)
Donor chamber	2.94 (1.77)	0.16 (0.08)	0.20 (0.15)
Total unabsorbed	52.3 (3.23)	53.2 (9.95)	53.0 (5.71)
<b>Total recovered</b>	<b>95.4 (2.89)</b>	<b>96.8 (1.49)</b>	<b>97.0 (0.49)</b>

During the initial 6-hour exposure period, penetration of  $^{14}\text{C}$ -methomyl from the 200 g/l undiluted concentrate was 33.1-fold and 4.9-fold faster through rat epidermis ( $190.1 \mu\text{g equiv}/\text{cm}^2/\text{hr}$ ) and rabbit skin, respectively, than through human epidermis ( $5.75 \mu\text{g equiv}/\text{cm}^2/\text{hr}$ ).



Table 6.87. Cumulative penetration of <sup>14</sup>C-methomyl from the undiluted concentrate

Time (hours)	Mean cumulative penetration (µg equiv/cm <sup>2</sup> )		
	Rat epidermis	Human epidermis	Rabbit skin
0.5	111.77 (14.55)	3.06 (0.64)	NA (NA)
1	251.20 (43.04)	6.11 (3.25)	2.24 (0.71)
2	542.17 (111.18)	8.23 (6.93)	12.36 (5.46)
4	951.73 (227.04)	22.37 (21.83)	83.23 (26.68)
6 <sup>a</sup> (end exposure)	1142.37 (288.74)	32.80 (29.60)	183.80 (51.82)
12 <sup>b</sup> (6 hours post-exposure)	1290.85 (348.67)	61.90 (29.60)	461.50 (133.93)
24 <sup>c</sup> (18 hours post-exposure)	1557.90 (NA)	54.50 (NA)	531.90 (NA)
<sup>a</sup> Penetration rate 0-6 hrs (µg equiv/cm <sup>2</sup> /hr)	190.1	5.75	39.0
<sup>b</sup> Penetration rate 6-12 hrs (µg equiv/cm <sup>2</sup> /hr)	24.1	7.57	44.1
<sup>c</sup> Penetration rate 12-24 hrs (µg equiv/cm <sup>2</sup> /hr)	1.71	2.19	13.8

Key: a) Mean for all exposures over 0-6 hours. b) Mean data for the 6 and 18 post-exposure groups. c) Mean data for the 18 hour post exposure group.

The recovery data for the undiluted concentrate are presented in Table 6.88. The total mean absorption was 78.6% (70.1-93.3%) for rat epidermis, 19.8% (8.1-35.9%) for human epidermis and 70.6% (62.1-82.9%) for rabbit skin. Total recovery ranged from 86.0-98.8% for the rat preparations, 96.3-121.2% for the human preparations and 87.2-94.6% for the rabbit preparations. There was a decrease in the radioactivity levels in the epidermal and skin preparations at 18 hours post exposure.

Table 6.88. Summary of the recovery data for the undiluted concentrate

Parameter	Percent of the applied dose)		
	(0 hour post-exposure)	(6 hour post exposure)	(18 hour post-exposure)
Rat epidermis			
Absorbed dose:			
Receptor fluid	65.7 (26.4)	60.5 (6.38)	90.2 (1.97)
Epidermis	6.67 (4.98)	9.60 (5.87)	3.02 (1.50)
Total absorbed	72.3 (23.4)	70.1 (8.37)	93.3 (2.29)
Unabsorbed:			
Epidermal wash	14.8 (18.7)	13.1 (9.39)	5.31 (2.05)
Donor chamber	1.64 (1.16)	2.75 (1.66)	0.28 (0.12)
Total unabsorbed	16.4 (18.9)	15.9 (7.98)	5.59 (2.09)
<b>Total recovered</b>	<b>88.8 (5.65)</b>	<b>86.0 (5.61)</b>	<b>98.8 (2.95)</b>
Human epidermis			
Absorbed dose:			
Receptor fluid	3.79 (2.11)	5.54 (2.64)	3.16 (1.22)
Epidermis	11.7 (11.9)	30.3 (29.7)	4.98 (3.56)
Total absorbed	15.5 (11.2)	35.9 (31.7)	8.13 (4.62)
Unabsorbed:			
Epidermal wash	76.4 (13.0)	69.5 (18.5)	112.4 (18.1)
Donor chamber	4.32 (4.27)	3.60 (2.77)	0.68 (0.24)
Total unabsorbed	80.8 (8.73)	73.1 (17.0)	113.1 (18.1)
<b>Total recovered</b>	<b>96.3 (2.47)</b>	<b>109.0 (18.1)</b>	<b>121.2 (14.4)</b>
Rabbit skin			
Absorbed dose:			
Receptor fluid	9.12 (1.81)	32.2 (10.8)	30.8 (14.5)
Skin	57.6 (8.24)	50.7 (9.58)	31.3 (2.86)
Total absorbed	66.7 (9.89)	82.9 (3.18)	62.1 (15.8)
Unabsorbed:			
Skin wash	19.0 (11.3)	10.9 (4.02)	30.3 (16.0)
Donor chamber	1.46 (0.68)	0.77 (0.48)	0.40 (0.19)
Total unabsorbed	20.4 (11.0)	11.7 (3.99)	30.8 (16.0)
<b>Total recovered</b>	<b>87.2 (4.17)</b>	<b>94.6 (1.04)</b>	<b>92.8 (5.37)</b>

Exclusive of the preparation and concentration tested, the penetration of radiolabelled methomyl continued following washing of the skin surface at 6 hours. An overall decrease in the radioactivity retained in the skin was accompanied by a corresponding increase in radioactivity in the receptor compartment. The company stated that 'since the *in vitro* dermal model ensured sink conditions for methomyl, penetration of radioactivity from the skin following washing can be considered an artefact of the test system, which does not occur *in vivo*' (see section B.6.12.2).

## Conclusions

The results obtained in this study demonstrate that the penetration and absorption of methomyl from the Methomyl 20SL formulation, when applied as an aqueous dilution or as an undiluted concentrate, was greatest for rat epidermis compared to human epidermis. However, it should be noted that there is considerable variability in the data from the different groups.

For the rat and the rabbit preparations, the mean absorption increases with concentration based on the percent of the dose absorbed (Table 6.89). For the human preparations, the mean absorption of the undiluted concentrate decreases or at worst remains the same as the aqueous dilution (note upper limit of the range). This *in vitro*

data indicates that at least a proportion of the radioactivity in the epidermal and skin preparations is systemically available.

Table 6.89. Summary of the mean absorption data (0-, 6- and 18-hour post exposure groups) for the aqueous dilution and the undiluted concentrate

Material tested	Mean percent of dose absorbed (receptor fluid + epidermal values)		
	Rat epidermis	Human epidermis	Rabbit skin
1.5 g/l aqueous dilution	52.8% (48.5-57.1%) <sup>a</sup>	29.7% (22.3-39.3%)	43.6% (43.1-44.0%)
200 g/l undiluted concentrate	78.6% (70.1-93.3%)	19.8% ( <sup>b</sup> 8.1-35.9%)	70.6% (62.1-82.9%)
	Mean percent of dose absorbed (receptor fluid only)		
	Rat epidermis	Human epidermis	Rabbit skin
1.5 g/l aqueous dilution	43.3% (37.7-46.9%)	13.6% (5.45-24.0%)	43.6% (2.65-15.4%)
200 g/l undiluted concentrate	72.1% (60.5-90.2%)	4.2% (3.16-5.54%)	24% (9.12-32.2%)

Key: a) Range. b) Note the high value obtained for the epidermal wash, the low value obtained for the donor chamber and the high total recovery.

Based on the mean percent of the radioactivity absorbed from the aqueous dilution (receptor fluid + epidermal value), rat epidermis was 1.8 (1.2-2.4) times more permeable than human epidermis. When absorption is based on the mean receptor fluid values, rat epidermis was approximately 3.2 (1.9-6.9) times more permeable than human epidermis. Based on the penetration rate (0-6 hours), rat epidermis was 4 times more permeable than human epidermis.

Based on the mean percent of the radioactivity absorbed from the undiluted concentrate (receptor fluid + epidermal value), rat epidermis was 4.0 (2-11.5) times more permeable than human epidermis. When absorption is based on the mean receptor fluid values alone, rat epidermis was approximately 17.2 (10.8-28.5) times more permeable than human epidermis. Based on the penetration rate (0-6 hours), rat epidermis was 33 times more permeable than human epidermis.

(Fasano, 2001a)

#### B.6.12.2 *In vivo*

<b>Report</b>	Fasano W.J. (2001b). Methomyl (DPX-X1179) 20SL: <i>in vivo</i> dermal absorption of [ <sup>14</sup> C]methomyl in the rat. Unpublished DuPont Report No.: DuPont-5697 (in-life phase: February 2001 to July 2001 ).
<b>Test facility</b>	DuPont Haskell Laboratory, P.O. Box 50, Elkton Road, Newark, Delaware, 19714 USA
<b>Guidelines</b>	OECD Guidelines for the Testing of Chemicals (Draft Guideline 427), Skin Absorption: <i>In Vivo</i> Method (December 2000).
<b>Deviations from guidelines</b>	Not applicable
<b>GLP</b>	Yes
<b>Non labelled methomyl</b>	DPX-X1179-512, H22577, chemical purity >97%
<b>Radiolabelled methomyl</b>	HOTC 0555, H-22705-46, specific activity 42.96 µCi/mg, radiochemical purity >95%.
<b>Formulation blank</b>	H-24819
<b>Acceptable</b>	Yes

## Material and methods

This study examined the dermal absorption of  $^{14}\text{C}$ -methomyl in male Sprague-Dawley rats. Methomyl 20SL (a formulated product containing 20% methomyl) was applied as a 1.5 g methomyl/l aqueous dilution and as the undiluted concentrate at 200 g methomyl/l. The formulation concentrations and application rates were designed to mimic potential field-use exposures. Absorption was followed using  $^{14}\text{C}$ -methomyl, which was uniformly blended into the Methomyl 20SL formulation prior to application. Each rat was housed separately in an all-glass metabolism cage during the exposure and post-exposure phases. The test materials were applied at a rate of  $10\ \mu\text{l}/\text{cm}^2$  to a  $10.5\ \text{cm}^2$  shaved area on the dorso-lumbar region using a non-occlusive protective device. The formulated test substance was allowed to remain in contact with the skin for a total exposure period of 6 hours. The amount of methomyl applied was  $15\ \mu\text{g}/\text{cm}^2$  and  $2000\ \mu\text{g}/\text{cm}^2$  for the 1.5 g/l aqueous dilution and the 200 g/l undiluted concentrate, respectively. At the completion of the exposure period, the application site for all rats was washed with a 2% soap solution and dried using natural sponges. Following washing, one group of four rats was sacrificed (0 hours post-exposure). The remaining rats were held in metabolism cages with groups of four rats each being sacrificed at 6-, 18-, and 66-hours post-exposure to monitor clearance of the absorbed dose and depletion of skin residues. Urine, faeces,  $^{14}\text{C}$ -organic volatiles and  $^{14}\text{CO}_2$  were collected during the exposure and post-exposure periods. Whole blood, the application skin site, non-dosed skin, carcass, residual feed and cage washings were collected from all animals at sacrifice. All samples were analysed using liquid scintillation counting and the measures of radioactivity were expressed as methomyl equivalents or as a percentage of the applied dose. Only the results expressed as a percentage of the applied dose are presented in the following evaluation.

Pilot studies were carried out to investigate elimination via the inhalation route and the systemic availability of radioactivity in the skin sites following a six-hour exposure and a 66-hour recovery. Following a single topical dose of 1.5 g/l aqueous solution, more than 9% of applied dose was eliminated as  $^{14}\text{C}$ -organic volatiles and  $^{14}\text{CO}_2$ . Therefore, these samples were collected for the 1.5 g/l aqueous solution in the main study. Following a single topical dose of 200 g/l undiluted concentrate, less than 2.5% of applied dose was eliminated as  $^{14}\text{C}$ -organic volatiles and  $^{14}\text{CO}_2$ .

Table 6.90. Experimental design

Group	Nominal methomyl concentration (g/l)	Nominal dose level ( $\mu\text{g}$ methomyl/ $\text{cm}^2$ )	<sup>a</sup> No of rats	Target ( $\mu\text{Ci}/\text{rat}$ )
A	1.5	15	16	7
B	200	2000	16	10

Key: a) Four rats sacrificed at 0, 6, 18 and 66 hours post exposure.

## Results

The analytical data for the dosing solutions are presented in Table 6.91.

Table 6.91. Summary of the dose solution concentrations

Group	Nominal methomyl concentration (g/l)	Verified concentration (g/l)	Specific activity of <sup>14</sup> C-methomyl (μCi/mg)
A	1.5	1.61	41.6
B	200	214.6	0.449

A summary of the absorption and body weight data are presented in Table 6.92.

Table 6.92. Summary of the mean absorption data following a single 6-hour exposure of a single topical application of the 1.5 g/l aqueous dilution

Group A	1.5 g/l aqueous dilution			
Body weight (g)	269.1 (6.86)	267.5 (6.40)	<sup>c</sup> 179.6 (6.40)	237 (14.5)
Absorbed dose (% of dose)				
Hours post exposure	0 (n = 4)	6 (n = 4)	18 (n = 4)	66 (n = 4)
Urine 0	2.22 (1.00) <sup>a</sup>	1.61 (0.96)	0.50 (0.13)	1.38 (0.32)
0-6		1.38 (0.41)	1.63 (0.54)	1.64 (0.36)
6-18		2.98 (1.20)	1.57 (3.69)	1.16 (0.42)
18-42			3.69 (1.10)	0.89 (0.30)
42-66				0.27 (0.07)
Total				5.32 (1.18)
Faeces	0.08 (0.08)	0.12 (0.05)	0.27 (0.11)	0.65 (0.24)
<sup>14</sup> C-organic volatiles (methanol trap)	0.27 (0.19)	0.61 (0.18)	1.35 (0.50)	0.92 (0.50)
<sup>14</sup> CO <sup>2</sup> (4N NaOH trap)	2.16 (0.83)	2.75 (1.36)	1.76 (1.86)	4.99 (1.13)
Residual feed	<sup>b</sup> NA (NA)	NA (NA)	0.13 (0.02)	0.20 (0.09)
Cage wash	1.44 (0.75)	1.31 (0.51)	1.02 (0.66)	NA (NA)
Dosed skin	27.30 (5.77)	34.21 (11.28)	57.88 (7.13)	39.34 (3.67)
Non-dosed skin	NA (NA)	NA (NA)	NA (NA)	NA (NA)
Carcass	7.76 (2.07)	5.43 (1.55)	5.24 (1.02)	4.14 (0.36)
Whole blood	0.87 (0.25)	0.94 (0.35)	1.06 (0.17)	1.15 (0.18)
RBC	0.33 (0.07)	0.33 (0.10)	0.42 (0.10)	0.42 (0.09)
Plasma	0.02 (0.1)	0.01 (0.01)	0.01 (0.00)	0.01 (0.01)
<b>Total absorbed</b>	<b>42.42 (10.02)</b>	<b>48.68 (7.03)</b>	<b>72.76 (5.00)</b>	<b>57.14 (6.39)</b>
Unabsorbed dose (% of dose)				
Body wrap	0.39	0.38	0.32	0.34
Mesh cover	1.38	0.47	0.19	0.23
Skin wash	44.74	45.96	22.02	38.44
O-ring	6.13	0.70	0.87	0.47
<b>Total unabsorbed</b>	<b>52.65 (9.13)</b>	<b>47.50 (6.22)</b>	<b>23.40 (5.85)</b>	<b>39.48 (6.89)</b>
<b>Total dose recovered</b>	<b>95.06 (1.90)</b>	<b>96.18 (1.83)</b>	<b>96.16 (3.11)</b>	<b>96.62 (0.88)</b>

Key: a) Standard deviation. b) Samples were below the limit of detection or limit of quantitation. c) 24-23% difference in mean body weight compared to other groups (range 171-190 g).

Following exposure to a single topical application of the 1.5 g/l aqueous dilution, an average of 55.25% of the applied dose had been absorbed by 6 hours (mean of 4 groups and includes skin radioactivity). An increase in the amount of radioactivity eliminated via the urine and faeces was accompanied by a related decrease in the amount of radioactivity associated with the remaining carcass. Depletion of radioactivity from the dosed skin was not observed up to 66 hours post-exposure. This observation is supported by data from the 18-hour post-exposure group, where, although inefficient washing of the skin resulted in a significantly greater portion of

the applied dose associated with the applied skin at the end of the 6-hour exposure period, a corresponding spike in the amount of radioactivity associated with the carcass or eliminated via the urine and faeces was not observed. These data suggest that the systemically-absorbed portion of the absorbed dose was no greater than the difference between the average total dose that had been absorbed by 6 hours (55.25%) and the average amount retained in the applied skin (39.68%) which is approximately 15.57% (based on the means of all four groups). The relatively high percentages of the applied dose retained in the skin do not appear to be systemically available. This observation is supported by the urinary excretion profile over the 18-42 and 42-66 post-exposure periods.

Table 6.93. Summary of the mean absorption data following a single 6-hour exposure of a single topical application of the 200 g/l undiluted concentrate

<b>Group A</b>	<b>200 g/l undiluted concentrate</b>			
Body weight (g)	226.1 (12.5)	223.9 (2.24)	229.7 (7.18)	224.5 (9.05)
<b>Absorbed dose (% of dose)</b>				
Hours post exposure	0 (n = 4)	6 (n = 4)	18 (n = 4)	66 (n = 4)
Urine 0	0.91 (0.58)	0.99 (0.35)	1.41 (0.78)	1.18 (0.71)
0-6		0.76 (0.25)	0.60 (0.32)	1.07 (0.53)
6-18		1.75 (0.59)	0.95 (0.79)	0.70 (0.39)
18-42			2.96 (1.22)	0.44 (0.17)
42-66				0.20 (0.07)
Total				3.33 (1.14)
Faeces	0.04 (0.02)	0.23 (0.21)	0.26 (0.24)	0.31 (0.10)
Residual feed	0.11 (0.04)	0.19 (0.13)	0.19 (0.11)	0.11 (0.02)
Cage wash	1.27 (0.17)	1.43 (0.56)	1.37 (0.48)	0.79 (0.37)
Dosed skin	6.82 (2.87)	6.63 (1.38)	5.92 (1.78)	6.09 (3.55)
Non-dosed skin	NA (NA)	NA (NA)	NA (NA)	NA (NA)
Carcass	1.35 (0.24)	1.18 (0.23)	1.41 (0.34)	1.22 (0.32)
Whole blood	0.16 (0.04)	0.20 (0.03)	0.27 (0.06)	0.22 (0.06)
RBC	0.05 (0.01)	0.07 (0.01)	0.09 (0.02)	0.08 (0.01)
Plasma	0.01 (0.01)	0.01 (0.01)	0.01 (0.01)	0.00 (0.00)
<b>Total absorbed</b>	<b>10.65 (3.79)</b>	<b>11.62 (1.55)</b>	<b>12.36 (3.01)</b>	<b>12.13 (4.55)</b>
<b>Unabsorbed dose (% of dose)</b>				
Body wrap	0.06 (0.01)	0.09 (0.07)	0.32 (0.38)	0.44 (0.49)
Mesh cover	0.20 (0.31)	0.26 (0.32)	0.65 (0.07)	0.01 (0.00)
Skin wash	87.11 (6.11)	79.33 (1.87)	76.39 (3.88)	75.58 (75.58)
O-ring	1.26	1.98 (1.35)	2.82 (1.17)	2.42 (2.43)
<b>Total unabsorbed</b>	<b>88.61 (5.92)</b>	<b>81.64 (0.60)</b>	<b>79.77 (4.06)</b>	<b>78.44 (5.31)</b>
<b>Total dose recovered</b>	<b>99.26 (2.72)</b>	<b>93.26 (1.31)</b>	<b>92.13 (1.59)</b>	<b>90.57 (1.74)</b>

Key: a) Standard deviation. b) Samples were below the limit of detection or limit of quantitation.

Following exposure to a single topical application of the 200 g/l undiluted concentrate, an average of 11.69% (means of 4 groups) of the applied dose had been absorbed by 6 hours. The amount of radioactivity eliminated via the urine and faeces increased with recovery time. However, the amount of radioactivity associated with the carcass and remained fairly similar with recovery time. Depletion of radioactivity from the dosed skin was not observed up to 66 hours post-exposure. These data suggest that the systemically available portion of the absorbed dose was no greater than the difference between the average total dose that had been absorbed by 6 hours (11.69%) and the average amount retained in the applied skin (6.37%) which is approximately 5.33%. However, the total recovery values for the 6-, 18- and 66-hour post-exposure groups

are slightly lower than those obtained for the aqueous dilutions. Approximately 1-9% of the radioactivity was not recovered for the undiluted concentrate. This might be related to the elimination of radioactivity via the inhalation route (not evaluated for the undiluted concentrate).

### Conclusions

Following exposure to a single topical application of the 1.5 g/l aqueous dilution and the 200 g/l undiluted concentrate of the Methomyl 20SL formulation, 55.3% and 11.7% of the applied dose was absorbed by 6 hours, respectively. Of the total percent absorbed, a significant portion was contained in the dosed skin at the end of the 6-hour exposure period. However, depletion of the absorbed radioactivity from the application skin site (39.68 for the aqueous dilution and 6.37% for the undiluted concentrate) was not observed up to 66 hours post-exposure, exclusive of formulation concentration tested. Based on the total absorbed dose data and lack of evidence for depletion of radioactivity from the dose site, the percent of applied dose that was systemically available was approximately 15.6% and 5.33% for the 1.5 g/l aqueous dilution and 200 g/l undiluted concentrate, respectively. To compensate for the absence of inhalation data for the undiluted concentrate, a value of 2.5% (obtained from pilot study) can be added on to the percent of the applied dose that was systemically available. This would give an estimated value of 7.83% of the applied dose for the undiluted concentrate.

(Fasano, 2001b)

#### B.6.12.3 Summary of the dermal absorption data

The dermal penetration of methomyl was measured *in vitro* and *in vivo*. Radiolabelled methomyl was blended with Methomyl 20SL (a formulated product containing 20% methomyl) and applied as either the undiluted concentrate (200 g/l) or as a 133-fold aqueous dilution (1.5 g/l). The concentrations and application rates were designed to mimic potential field-use exposures. The relative dermal penetration and absorption of methomyl *in vitro* was in the following order: rat > rabbit > human.

The *in vivo* data in male rats indicates that 15.6% of the aqueous dilution and 7.83% of undiluted concentrate would be systemically available (adjusted to compensate for the absence of inhalation data) following a six hour dermal exposure and an extensive recovery period. These absorption values exclude the radioactivity in the dosed skin because depletion of radioactivity from the dosed skin was not observed for up to 66 hours post exposure (i.e. was considered to be unavailable systemically).

Where *in vivo* (rat) and *in vitro* dermal absorption data (rat and human epidermis) are available, the *in vitro* data is used to estimate the permeability differences for rat and human skin for the different test materials. This would result in correction or conversion factors which are then used to adjust the *in vivo* rat data to account for any differences in permeability between rat and human skin.

Since the *in vivo* data indicate that the skin residues were not systemically available, it seems reasonable to use the *in vitro* receptor fluid values to estimate the permeability differences between rat and human skin. These *in vitro* data indicate that rat skin is approximately 1.9-6.9 and 10.8-28.5 times more permeable than human skin to the

aqueous dilution and concentrate, respectively. Therefore, adopting a precautionary approach, values of 10% and 1.0% can be proposed for the aqueous dilution and the concentrate, respectively, for the operator risk assessments.

#### **B.6.13 Toxicology of non-active substances**

The available information on the non-active substances present in 'Methomyl 20L' ('Methomyl 20SL') does not indicate any toxicological concerns that would not be investigated in the routine acute toxicity tests performed.



**B.6.14 EXPOSURE DATA (IIIA 7.2)**

The use of a single product has been supported in this Review: that of 'Methomyl 20SL' (also known as 'Lannate 20L'), a SL formulation containing a nominal 200 g/l methomyl, as a horticultural insecticide on outdoor crops of cucumber, courgette, tomato, eggplant and grape (table and wine). Details of the representative uses are summarised in Table B.6.94.

Table B.6.94 Supported use of 'Methomyl 20SL'

<b>Use</b>	Horticultural insecticide on outdoor crops of cucumber, courgette, tomato, eggplant and grape (table and wine)
<b>Application method (proposed)</b>	Fruiting vegetables (field crops) - cucumber, courgette, eggplant, tomato : tractor-mounted/trailed field crop sprayer Grape: tractor-mounted/trailed broadcast air-assisted sprayer
<b>Maximum individual dose</b>	Cucumber, courgette, tomato, eggplant 1.25 to 2.25 litres of product/ha (0.25 to 0.45 kg methomyl/ha) Grape (medium volume) 1.75 litres of product/ha (0.35 kg methomyl/ha) Grape (high volume) 2.25 litres of product/ha (0.45 kg methomyl/ha)
<b>Application volume</b>	Cucumber, courgette, tomato, eggplant: 500 to 1000 litres of water/ha Grape (medium volume): 300 to 450 litres of water/ha Grape (high volume): >450 to 1200 litres of water/ha
<b>Maximum total dose</b>	Up to 2 applications <i>per annum</i>
<b>Interval between applications</b>	Cucumber, courgette, tomato, eggplant : minimum 14 days Grape: minimum 14 days
<b>Pre-harvest interval</b>	Cucumber, courgette, tomato, eggplant : 7 days before harvest Grape: 14 days before harvest
<b>Packaging</b>	1, 5 and 10 litre wide necked HDPE screw-top containers

Growers using 'Methomyl 20SL' as proposed are likely to apply the product on no more than a few days *per annum*, whereas contract operators might use the product for a few consecutive weeks. In both situations, operators will only experience short-term exposure (<90 days/year). As data indicate that humans are the most sensitive species to methomyl-induced cholinesterase inhibition, a systemic AOEL of 0.005 mg/kg bw/day is proposed in this evaluation, based on the NOAEL of 0.1 mg/kg bw/day for increased saliva secretion and RBC and plasma cholinesterase inhibition in an acute oral human volunteer study and using an assessment factor of 20 (to reflect the small group size and wide variations between individuals in the study) with no correction being required for oral absorption. This evaluation concludes that it is inappropriate to set a dermal or inhalation AOEL based on the dermal and inhalation studies submitted (see Section B.6.10.3).

Dermal absorption values for 'Methomyl 20SL' of 1% for the concentrate and 10% for the spray solutions are considered appropriate, based on the results of an *in vivo* rat study and a comparative *in vitro* dermal absorption study using this formulation (see Section B.6.12).

Evaluation of acute formulation toxicology data (see Section B.6.11) indicates that 'Methomyl 20SL' warrants the hazard classification 'Toxic' with the risk phrases 'Toxic if swallowed' (R25), 'Harmful by inhalation' (R20) and 'Risk of serious damage to eyes' (R41). On the basis of this hazard classification alone, it is appropriate for operators to wear suitable protective gloves (to reduce the likelihood of hand-to-mouth and hand-to-eye contamination) and face protection when handling the concentrate. Although 'Methomyl 20SL' is classified as harmful by inhalation on the basis of an acute study using the formulation presented as an aerosol (MMAD <3 µm), as methomyl has a moderate vapour pressure ( $7.2 \times 10^{-4}$  Pa at 25 °C), the level of inhalation exposure for an unprotected operator mixing and loading the SL formulation under normal field conditions is likely to be negligible.

#### **B.6.14.1 Operator exposure (IIIA 7.2.1)**

##### **B.6.14.1.1 Estimation of operator exposure (IIIA 7.2.1.1)**

The notifier has estimated the level of operator exposure to methomyl resulting from the use of 'Methomyl 20SL' on field (fruiting vegetable) crops and grapevines using the German model<sup>1</sup> and the UK Predictive Operator Exposure Model (POEM)<sup>2</sup>. The notifier's estimates reflect the use of the highest supported maximum individual dose of 2.25 litres of product/ha (450 g methomyl/ha) and the minimum water volume of 500 litres of spray solution/ha for field crops and 300 litres of spray solution/ha for grapevines (although it is noted from the GAP table that a water volume of >450 litres of spray solution/ha is proposed for use on grapevines when the maximum individual dose exceeds 1.75 litres of product/ha).

Operator exposure estimates, based on the parameters detailed in Table B.6.94, are presented in Appendix 4 and summarised in Tables B.6.95 to B.6.101. Although the notifier considered only the use of tractor-mounted/trailed sprayers, estimates reflecting the use of knapsack sprayers have also been calculated as this method of application may be used by small scale growers (however, the German model has no data on the use of knapsack sprayers on field crops and the UK POEM has no data on knapsack use on high crops). For field crops, the notifier has assumed a work rate of 10 ha/day (which is stated to reflect normal practice in southern Europe), however, no evidence has been provided to justify this and the standard work rates associated with each model have also been used. For the UK POEM estimates, a container size of 10 litres (45 mm closure) is assumed (which results in a similar level of operator contamination to the proposed 1 litre container and a higher level of operator contamination than the proposed 5 litre container when an equivalent amount of product is dispensed).

Table B.6.95 Operator exposure to methomyl resulting from the use of 'Methomyl 20SL' on grapevines (German model for orchard sprayers)

Dermal exposure mg/person/day		Inhalation exposure mg/person/day		Total systemic exposure *	
Mix/loading	Application	Mix/loading	Application	mg/kg bw/day**	% of AOEL
No PPE					
8.640	41.400	0.002	0.065	0.0613	1226%
Gloves when handling the concentrate					
0.086	41.400	0.002	0.065	0.0601	1202%
Gloves when handling the concentrate and during application					
0.086	38.905	0.002	0.065	0.05654	1131%
Gloves when handling the concentrate, coveralls and gloves during application					
0.086	6.073	0.002	0.065	0.00964	193%
* assuming a dermal absorption for methomyl of 1% for the concentrate and 10% for the spray solution ** assuming a body weight of 70 kg (default German model value) AOEL evaluator's proposed short-term systemic AOEL of 0.005 mg/kg bw/day					

Table B.6.96 Operator exposure to methomyl resulting from the use of 'Methomyl 20SL' on grapevines (UK POEM for orchard sprayers)

Dermal exposure mg/person/day		Inhalation exposure mg/person/day		Total systemic exposure *	
Mix/loading	Application	Mix/loading	Application	mg/kg bw/day**	% of AOEL
No PPE					
60.000 <sup>MV</sup> 80.000 <sup>HV</sup>	141.400 <sup>MV</sup> 121.200 <sup>HV</sup>	negligible	0.350 <sup>MV</sup> 0.300 <sup>HV</sup>	0.2515 <sup>MV</sup> 0.2203 <sup>HV</sup>	5030% <sup>MV</sup> 4406% <sup>HV</sup>
Gloves when handling the concentrate					
3.000 <sup>MV</sup> 4.000 <sup>HV</sup>	141.400 <sup>MV</sup> 121.200 <sup>HV</sup>	negligible	0.350 <sup>MV</sup> 0.300 <sup>HV</sup>	0.2420 <sup>MV</sup> 0.2077 <sup>HV</sup>	4840% <sup>MV</sup> 4154% <sup>HV</sup>
Gloves when handling the concentrate and contaminated surfaces					
3.000 <sup>MV</sup> 4.000 <sup>HV</sup>	99.400 <sup>MV</sup> 85.200 <sup>HV</sup>	negligible	0.350 <sup>MV</sup> 0.300 <sup>HV</sup>	0.1720 <sup>MV</sup> 0.1477 <sup>HV</sup>	3440% <sup>MV</sup> 2954% <sup>HV</sup>
* assuming a dermal absorption for methomyl of 1% for the concentrate and 10% for the spray solution ** assuming a body weight of 60 kg (default POEM value) AOEL evaluator's proposed short-term systemic AOEL of 0.005 mg/kg bw/day medium volume or high volume use (see Table B.6.82)					

Table B.6.97 Operator exposure to methomyl resulting from the use of 'Methomyl 20SL' on grapevines (German model for knapsack sprayers)

Dermal exposure mg/person/day		Inhalation exposure mg/person/day		Total systemic exposure *	
Mix/loading	Application	Mix/loading	Application	mg/kg bw/day**	% of AOEL
No PPE					
92.250	18.180	0.023	0.135	0.0414	828%
Gloves when handling the concentrate					
0.923	18.180	0.023	0.135	0.0284	568%
Gloves when handling the concentrate and during application					
0.923	13.458	0.023	0.135	0.0216	432%
Gloves when handling the concentrate, coveralls and gloves during application					
0.923	2.770	0.023	0.135	0.0063	126%
* assuming a dermal absorption for methomyl of 1% for the concentrate and 10% for the spray solution ** assuming a body weight of 70 kg (default German model value) AOEL evaluator's proposed short-term systemic AOEL of 0.005 mg/kg bw/day					

Table B.6.98 Operator exposure to methomyl resulting from the use of 'Methomyl 20SL' on grapevines (UK POEM for knapsack sprayers – based on data for low crops only)

Dermal exposure mg/person/day		Inhalation exposure mg/person/day		Total systemic exposure *	
Mix/loading	Application	Mix/loading	Application	mg/kg bw/day**	% of AOEL
No PPE					
400.000 <sup>MV</sup> 540.000 <sup>HV</sup>	119.000 <sup>MV</sup> 102.000 <sup>HV</sup>	negligible	0.140 <sup>MV</sup> 0.120 <sup>HV</sup>	0.2673 <sup>MV</sup> 0.2620 <sup>HV</sup>	5346% <sup>MV</sup> 5240% <sup>HV</sup>
Gloves when handling the concentrate					
20.000 <sup>MV</sup> 27.000 <sup>HV</sup>	119.000 <sup>MV</sup> 102.000 <sup>HV</sup>	negligible	0.140 <sup>MV</sup> 0.120 <sup>HV</sup>	0.2040 <sup>MV</sup> 0.1765 <sup>HV</sup>	4080% <sup>MV</sup> 3530% <sup>HV</sup>
Gloves when handling the concentrate and during application					
20.000 <sup>MV</sup> 27.000 <sup>HV</sup>	57.750 <sup>MV</sup> 49.500 <sup>HV</sup>	negligible	0.140 <sup>MV</sup> 0.120 <sup>HV</sup>	0.1019 <sup>MV</sup> 0.0890 <sup>HV</sup>	2038% <sup>MV</sup> 1780% <sup>HV</sup>
Gloves when handling the concentrate, impermeable coveralls and gloves during application					
20.000 <sup>MV</sup> 27.000 <sup>HV</sup>	21.880 <sup>MV</sup> 18.750 <sup>HV</sup>	negligible	0.140 <sup>MV</sup> 0.120 <sup>HV</sup>	0.0421 <sup>MV</sup> 0.0378 <sup>HV</sup>	843% <sup>MV</sup> 756% <sup>HV</sup>
* assuming a dermal absorption for methomyl of 1% for the concentrate and 10% for the spray solution ** assuming a body weight of 60 kg (default POEM value) AOEL evaluator's proposed short-term systemic AOEL of 0.005 mg/kg bw/day medium volume or high volume use (see Table B.6.82)					

Table B.6.99 Operator exposure to methomyl resulting from the use of 'Methomyl 20SL' on field crops (German model for field crop sprayers)

Dermal exposure mg/person/day		Inhalation exposure mg/person/day		Total systemic exposure *	
Mix/loading	Application	Mix/loading	Application	mg/kg bw/day**	% of AOEL
No PPE					
21.6 (10.8)	18.360 (9.180)	0.005 (0.003)	0.009 (0.005)	0.0295 (0.0148)	590% (295%)
Gloves when handling the concentrate					
0.216 (0.108)	18.360 (9.180)	0.005 (0.003)	0.009 (0.005)	0.0266 (0.0132)	529% (264%)
Gloves when handling the concentrate and during application					
0.216 (0.108)	14.974 (7.487)	0.005 (0.003)	0.009 (0.005)	0.0216 (0.0108)	433% (216%)
Gloves when handling the concentrate, coveralls and gloves during application					
0.216 (0.108)	1.294 (0.647)	0.005 (0.003)	0.009 (0.005)	0.0021 (0.0010)	42% (21%)
* assuming a dermal absorption for methomyl of 1% for the concentrate and 10% for the spray solution ** assuming a body weight of 70 kg (default German model value) AOEL evaluator's proposed short-term systemic AOEL of 0.005 mg/kg bw/day ( ) values in brackets are based on the notifier's assumed work rate of 10 ha/day					

Table B.6.100 Operator exposure to methomyl resulting from the use of 'Methomyl 20SL' on field crops (UK POEM for field crop sprayers)

Dermal exposure mg/person/day		Inhalation exposure mg/person/day		Total systemic exposure *	
Mix/loading	Application	Mix/loading	Application	mg/kg bw/day**	% of AOEL
No PPE					
240.000 (60.000)	37.395	negligible	0.054	0.1032 (0.0732)	2064% (1464%)
Gloves when handling the concentrate					
12.000 (3.000)	37.395	negligible	0.054	0.0652 (0.0637)	1304% (1274%)
Gloves when handling the concentrate and contaminated surfaces					
12.000 (3.000)	5.805	negligible	0.054	0.0126 (0.0111)	252% (222%)
* assuming a dermal absorption for methomyl of 1% for the concentrate and 10% for the spray solution ** assuming a body weight of 60 kg (default POEM value) AOEL evaluator's proposed short-term systemic AOEL of 0.005 mg/kg bw/day ( ) values in brackets are based on the notifier's assumed work rate of 10 ha/day					

Table B.6.101 Operator exposure to methomyl resulting from the use of 'Methomyl 20SL' on field crops (UK POEM for knapsack sprayers)

Dermal exposure mg/person/day		Inhalation exposure mg/person/day		Total systemic exposure *	
Mix/loading	Application	Mix/loading	Application	mg/kg bw/day**	% of AOEL
No PPE					
540.000	91.800	negligible	0.108	0.2448	4896%
Gloves when handling the concentrate					
54.000	91.800	negligible	0.108	0.1638	3276%
Gloves when handling the concentrate and contaminated surfaces					
54.000	44.550	negligible	0.108	0.0851	1701%
Gloves when handling the concentrate, impermeable coveralls and gloves during application					
54.000	16.875	negligible	0.108	0.0389	779%
* assuming a dermal absorption for methomyl of 1% for the concentrate and 10% for the spray solution ** assuming a body weight of 60 kg (default POEM value) AOEL evaluator's proposed short-term systemic AOEL of 0.005 mg/kg bw/day					

The German model estimates summarised above indicate that the use of 'Methomyl 20SL' on grapes through tractor-mounted/trailed equipment will result in an unacceptable level of systemic operator exposure to methomyl for a operator wearing protective gloves when handling the concentrate and coveralls and protective gloves during application (systemic exposure equivalent to 1.9x the proposed systemic AOEL of 0.005 mg/kg bw/day). The corresponding UK POEM estimates also indicate an unacceptable level of systemic exposure for an operator wearing protective gloves when handling the concentrate and during application (systemic exposure equivalent to 30x to 34x the proposed systemic AOEL).

German model estimates indicate that the use of 'Methomyl 20SL' on grapes through hand held equipment will result in an unacceptable level of systemic operator exposure to methomyl for a operator wearing protective gloves when handling the concentrate and coveralls and protective gloves during application (systemic exposure equivalent to 1.3x the proposed systemic AOEL of 0.005 mg/kg bw/day). The corresponding UK POEM estimates also indicate an unacceptable level of systemic exposure for an operator wearing protective gloves when handling the concentrate and impermeable coveralls and protective gloves during application (systemic exposure equivalent to approximately 8x the proposed systemic AOEL).

German model estimates indicate that the use of 'Methomyl 20SL' on field crops through tractor-mounted/trailed equipment will result in an acceptable level of systemic operator exposure to methomyl for a operator wearing protective gloves when handling the concentrate and coveralls and protective gloves during application (systemic exposure equivalent to 21% to 42% of the proposed systemic AOEL of 0.005 mg/kg bw/day). The corresponding UK POEM estimates indicate an unacceptable level of systemic exposure for an operator wearing protective gloves when handling

the concentrate and when handling contaminated surfaces (systemic exposure equivalent to 2.2x to 2.5x the proposed systemic AOEL).

UK POEM estimates indicate that the use of 'Methomyl 20SL' on field crops through hand held equipment will result in an unacceptable level of systemic operator exposure to methomyl for a operator wearing protective gloves when handling the concentrate and impermeable coveralls and protective gloves during application (systemic exposure equivalent to approximately 8x the proposed systemic AOEL of 0.005 mg/kg bw/day). Corresponding German model data for the use of hand-held sprayers on field crops are not available.

#### **B.6.14.1.2 Measurement of operator exposure (IIIA 7.2.1.2)**

An operator exposure (dosimetry) study was submitted by the notifier to quantify the levels of dermal and inhalation exposure to methomyl resulting from the representative use of 'Lannate 20L' ('Methomyl 20SL') on grapes. Monitoring took place at 12 locations in the main grape growing regions of southern France from 23 July to 2 August, 2001 and the field and laboratory phases of the study were conducted in accordance with OECD guidelines and GLP.

The product (supplied to the study participants in 5 litre packs) was mixed and loaded by 12 operators and applied by 12 different operators, all experienced in the use of pesticides. The product was applied at a target dose of 2.25 litres of product/ha (450 g methomyl/ha), the maximum supported application rate, in a volume of 300 litres of spray solution/ha (i.e. a lower volume than that proposed in the GAP table for this application rate). A range of tractor-mounted, trailed and self-propelled broadcast air-assisted sprayers were used with most being ducted 'cannon' type machines commonly used in vineyards. Sprayer tank capacity ranged from 300 litres to 1000 litres and all sprayers were loaded through the top of the tank. Three sprayers were operated with no cab, 7 with an open cab, 1 with a closed cab without a pesticide filter and 1 with a closed cab with carbon filtration.

Details of the study subjects and work rate information is presented in Table B.6.102. This information indicates that tasks performed by all study subjects represent a full day's work and the study results do not require correction on the basis of duration of exposure.

Table B.6.102. Study subject details and work rate information.

Site	Subject *	Body weight (kg)	Amount of a.s. handled (kg)	Number of fills	Area treated (ha)	Exposure time clothing (hours:mins)	Exposure time filters (mins)	Total activity time (mins)
1	1 (a)***	91	-	-	6.0	294	295	294
	2 (m)	70	2.70	3	-	252	31	28
2	3 (a)	80	-	-	5.2	305	292	260
	4 (m)***	91	2.40	3	-	262	22	17
3	5 (a)	74	-	-	6.0	300	297	242
	6 (m)**	75	2.70	5	-	310	41	37
4	7 (a)	86	-	-	6.8	258	253	233
	8 (m)	132	3.00	4	-	300	57	41
5	9 (m)	90	3.38	3	-	329	40	48
	10 (a)	80	-	-	7.5	410	347	318
6	11 (a)	100	-	-	8.4	335	316	245
	12 (m)	87	3.60	3	-	279	44	43
7	13 (a)	82	-	-	8.0	247	261	267
	14 (m)	78	3.60	3	-	232	40	33
8	15 (a)	72	-	-	7.6	290	279	199
	16 (m)	77	3.60	3	-	218	31	27
9	17 (a)	70	-	-	7.0	330	249	247
	18 (m)	95	3.15	7	-	293	60	60
10	19 (m)	62	3.60	3	-	252	19	30
	20 (a)	79	-	-	8.0	348	309	238
11	21 (a)	89	-	-	6.0	276	219	169
	22 (m)	83	3.00	3	-	248	29	32
12	23 (a)	100	-	-	5.2	330	285	336
	24 (m)	88	3.75	3	-	271	20	20

\* m = mixer/loader, a = applicator;

\*\* female study participant, all other subjects were male

\*\*\* applicator '1' and mixer '4' were the same individual (performing different tasks)

Dermal exposure was measured using whole-body dosimeters (pre-washed cotton coverall without hood, a long sleeved polyester/viscose T-shirt, polyester/viscose long-johns and protective nitrile gloves), face/neck wipes (using 10 cm x 10 cm cotton gauze moistened with Aerosol OT-100 detergent solution) and hand washes (Aerosol OT-100). The study participants wore their own protective footwear and the mixer/loaders were provided with a face shield to wear when handling the concentrate. Inhalation exposure was measured using IOM inhalable particle samplers with GF/A filter papers mounted in each operator's breathing zone and attached to a personal air sampling pump with a calibrated flow rate of 2.0 l/min.

Weather conditions (wind speed and direction, air temperature, relative humidity and cloud cover) were monitored at each site for each mixing/loading operation and at approximately hourly intervals during application. All spraying was carried out under suitable conditions.

At the end of the trial period coveralls were cut into sections to produce separate samples for the arms, legs, front torso and rear torso. All other



matrices were used as whole samples. Inner and outer clothing samples, facial swabs and protective gloves (as a pair) were wrapped in aluminium foil, labelled and bagged. Air sampler filters were placed in sealed, labelled tubes and hand wash solutions were put in sealed, labelled containers. Immediately after packaging, all samples were stored at -20 °C until analysed. Samples were stored for at least 36 days before analysis, as detailed in Table B.6.103.

Table B.6.103 Treatment of samples.

Sample matrix	Days in transit	Interval between sampling and extraction (days)	Interval between sampling and quantification (days)
coverall legs	0-7	42-50	50-58
coverall arms	0-7	34-42	38-45
coverall front	0-7	44-52	55-61
coverall back	0-7	48-53	55-68
T-shirt	0-7	55-63	66-78
long johns	0-7	56-59	65-72
protective gloves	0-7	86-93	90-99
hand wash	0-7	40-48	42-51
face swab	0-7	34-42	36-46
air filters	0-7	62-71	73-83

Field and transport recovery samples for all media were produced at all sites at the start of each working day. Duplicate samples of inner and outer clothing matrices and protective gloves were spiked and exposed to ambient conditions at a location near the trial fields for the duration of the working day (field recovery samples) or packaged and frozen immediately after fortification (transport recovery samples). Duplicate air filters were spiked and connected to personal air sampling pumps for a period of at least 240 minutes (field recovery samples) or packaged and frozen immediately after fortification (transport recovery samples). Duplicate untreated control samples were exposed to ambient conditions in the same way as the corresponding field recovery samples.

Samples of coveralls (1000 cm<sup>2</sup>) and undergarments (1000 cm<sup>2</sup>) were extracted using acetone. Samples of the hand wash solution (a 200 ml aqueous solution of 0.01% sodium dioctyl sulfosuccinate) were extracted using solvent partition with methylene chloride. Face/neck wipes (a pair of 10 cm x 10 cm cotton gauze samples each moistened with 8 ml of an aqueous solution of 0.01% sodium dioctyl sulfosuccinate) were extracted using methylene chloride. Protective glove samples (pair) were extracted using a water/acetonitrile (80%:20%) mixture and then purified by solvent partition using methylene chloride. Air sampling media (single filter papers) were extracted using acetone.

The extracts were analysed using HPLC with post-column derivatisation and fluorescence detection. All results were calculated using linear regression from external standards.

Validation data, which are presented in Table B.6.104, are considered to be acceptable.

Salvi, M. (2002)

Table B.6.104 Summary of analytical method and validation for operator exposure study samples

Substrate	Analyte	Limit of quantification (µg/sample)	Fortification level (µg/sample)	Mean recovery (%) (range %)	Repeatability (% RSD)	Linearity demonstrated	Interference
Coveralls (1000 cm <sup>2</sup> )	Methomyl	10.0	10.0 100.0 20000.0	100 (95-106) (n = 8) 102 (99-105) (n = 5) 110 (106-114) (n = 5)	5.6 (n = 8) 2.6 (n = 5) 3.6 (n = 5)	Yes (r >0.99)	None
Inner garments (1000 cm <sup>2</sup> )	Methomyl	2.0	2.0 20.0 200.0	91 (88-94) (n = 8) 93 (89-98) (n = 5) 108 (106-110) (n = 5)	3.5 (n = 8) 4.8 (n = 5) 1.8 (n = 5)	Yes (r >0.99)	None
Protective gloves (pair)	Methomyl	20.0	20.0 200.0 40000.0	92 (89-95) (n = 8) 95 (92-98) (n = 5) 89 (84-94) (n = 5)	3.0 (n = 8) 3.2 (n = 5) 5.8 (n = 5)	Yes (r >0.99)	None
Face/neck wipe (2 wipes)	Methomyl	2.0	2.0 20.0 400.0	93 (88-98) (n = 8) 99 (98-100) (n = 5) 95 (88-102) (n = 5)	5.8 (n = 8) 1.4 (n = 5) 7.8 (n = 5)	Yes (r >0.99)	None
Hand wash (200 ml)	Methomyl	2.0	2.0 20.0 200.0	91 (82-100) (n = 5) 98 (91-105) (n = 5) 90 (88-92) (n = 5)	9.8 (n = 5) 7.4 (n = 5) 2.7 (n = 5)	Yes (r >0.99)	None
Air filter (1 paper)	Methomyl	1.0	1.0 10.0 100.0	88 (83-93) (n = 5) 101 (98-104) (n = 5) 88 (74-102) (n = 5)	5.9 (n = 5) 3.3 (n = 5) 16.0 (n = 5)	Yes (r >0.99)	None

Field and storage recovery results are shown in Table B.6.105.

Table B.6.105 Results from field and storage recovery experiments.

Matrix	LOQ (µg)	Fortification levels	Field samples		Storage samples	
			Total replicates	Mean recovery (%)	Total replicates	Mean recovery (%)
outer clothing	10	100x and 2000x LOQ	28	98 ±9.7%	14	105 ±9%
inner clothing	2	10x and 100x LOQ	28	94 ±15%	14	97 ±13%
protective gloves	20	200x and 2000x LOQ	28	86 ±6.7%	14	81 ±14%
hand wash	2	10x and 100x LOQ	-	-	14	99 ±11%
face/neck wipe	2	20x and 200x LOQ	-	-	14	78 ±10%
air filter	1	10x LOQ	14	103 ±15%	7	100 ±4.6%
		100x LOQ	14	64 ±25%	7	114 ±10%

For all matrices at each fortification level, the mean recoveries were in the range of 70% to 110% with a standard deviation of less than 20% with the exception of air sampling media fortified at 0.10 mg/sample (100x LOQ) which produced a mean recovery of 64% ± 25%). However, as the range of air filter residues measured in the operator monitoring study (see Tables B.6.106 and B.6.107) was similar to the low fortification level (for which recovery was adequate), these field and storage recovery results are considered satisfactory.

Reported exposure levels for study subjects involved in mixing and loading operations are presented in Table B.6.106, and those for sprayer operators are presented in Table B.6.107.

Table B.6.106. Residues detected on sampling matrices during mixing and loading tasks.

Test subject	Residue detected µg/person/day									
	Outer clothing				Prot'ive gloves	Inner clothing		Detergent washes		Air filter
	Legs	Arms	Front	Back		T-shirt	Long Js	Face	Hands	
2 <sup>a</sup>	1200	1100	2000	93	18000	3.7	<2	<2	4	<1
4 <sup>bcd</sup>	840	1800	1300	110	42000	13	3.8	<2	26	<1
6 <sup>c</sup>	17000	810	1400	210	32000	17	38	4.1	450	<1
8 <sup>e</sup>	380	<10	380	30	1500	<2	<2	<2	3.9	<1
9 <sup>a</sup>	970	290	540	130	12000	12	3.4	2.8	6.8	<1
12 <sup>e</sup>	900	430	1500	41	46000	9.4	4.9	5.1	90	<1
14 <sup>e</sup>	110	290	220	38	3900	3.1	3.2	<2	3.4	<1
16 <sup>a</sup>	75000	600	18000	720	32000	8	13	<2	37	<1
18 <sup>a</sup>	360	1200	510	19	49000	6.7	2.2	5	8.9	<1
19 <sup>f</sup>	730	2000	1800	410	96000	70	82	29	670	<1
22	180	930	800	29	22000	3	<2	<2	5.9	1
24 <sup>a</sup>	900	1300	630	70	19000	5.7	71	<2	21	1

<sup>a</sup> operator climbed/leant onto sprayer during filling<sup>b</sup> operator opened tank without protective gloves<sup>c</sup> operator walked through treated crop<sup>d</sup> operator touched chemical container without gloves<sup>e</sup> operator touched coveralls with protective gloves<sup>f</sup> operator removed foil container seal with protective glovesTable B.6.107. Residues detected on sampling matrices during application.

Test subject	Residue detected µg/person/day									
	Outer clothing				Prot'ive gloves	Inner clothing		Detergent washes		Air filter
	Legs	Arms	Front	Back		T-shirt	Long Js	Face	Hands	
1 <sup>ao</sup>	2200	1100	1200	650	640	91	11	23	330	5.2
3 <sup>bf</sup>	260	150	74	180	32	13	2.2	<2	110	2.7
5 <sup>o</sup>	2700	1700	910	2700	410	80	20	17	6400	3.2
7 <sup>cfz</sup>	20	12	22	10	32	3	<2	<2	11	<1
10 <sup>adn</sup>	2800	1900	1700	1600	660	140	14	24.2	260	16
11 <sup>eo</sup>	7000	6300	5700	5000	2800	160	57	28	1100	26
13 <sup>cn</sup>	4800	8000	3100	1400	2500	220	42	24	680	9
15 <sup>cn</sup>	10000	8500	5900	4700	3300	340	39	20	1400	40
17 <sup>o</sup>	6450	5100	5700	5600	4900	180	49	58	810	25
20 <sup>aco</sup>	3500	3300	3400	760	8700	210	36	8700	320	13
21 <sup>o</sup>	6000	4100	3900	3200	3600	400	40	3600	560	21
23 <sup>o</sup>	4600	3400	3500	850	98	85	13	98	460	11

<sup>a</sup> operator climbed onto sprayer to check tank contents.<sup>b</sup> operator entered newly treated crop when checking sprayer<sup>c</sup> operator touched the sprayer without wearing protective gloves.<sup>d</sup> operator used an airflow helmet during application (face exposure value is total of face wipe and visor wipe)<sup>e</sup> operator sat on protective gloves when driving<sup>f</sup> tractor with closed cab used<sup>o</sup> tractor with open cab used<sup>n</sup> tractor with no cab used<sup>z</sup> tractor cab equipped with pesticide filter

The levels of Potential Dermal Exposure (total residues outer clothing, protective gloves, inner clothing, face/neck wash and hand wash), Actual Dermal Exposure

(total residues on inner clothing, face/neck wash and hand wash) and inhalation exposure (residues on air filters corrected to reflect a breathing rate of 29 litres/minute) are summarised in Table B.6.108. A value of half of the LOQ has been assigned to samples with residue levels detected at below the LOQ for that matrix. These values have been expressed in terms of mg/kg bw/day using the individual body weights of the study subjects.

Table B.6.108 Summary of Potential Dermal Exposure, Actual Dermal Exposure and inhalation exposure.

Operator exposure mg/kg bw/day									
	Potential Dermal Exposure			Actual Dermal Exposure			Inhalation exposure		
	Mixing	Spraying	Total	Mixing	Spraying	Total	Mixing	Spraying	Total
Minimum	0.0174	0.0013 (0.0686)	0.0187 (0.0861)	0.0001	0.0002 (0.0050)	0.0002 (0.0051)	0.0001	0.0001 (0.0006)	0.0001 (0.0007)
Arithmetic mean	0.5561	0.2167 (0.2589)	0.7727 (0.8149)	0.0021	0.0285 (0.0340)	0.0306 (0.0361)	0.0001	0.0026 (0.0030)	0.0027 (0.0031)
Geometric mean	0.3174	0.1123 (0.2228)	0.4297 (0.5402)	0.0005	0.0105 (0.0189)	0.0110 (0.0195)	0.0001	0.0016 (0.0023)	0.0017 (0.0024)
75 <sup>th</sup> percentile	0.5953	0.3056 (0.3460)	0.9009 (0.9413)	0.0012	0.0317 (0.0450)	0.0328 (0.0462)	0.0001	0.0035 (0.0037)	0.0036 (0.0038)
Maximum	1.6418	0.4750 (0.4750)	2.1168 (2.1168)	0.0137	0.1173 (0.1173)	0.1310 (0.1310)	0.0002	0.0081 (0.0081)	0.0082 (0.0082)
( ) values in brackets exclude those operators using closed cabs (subjects 3 and 7)									

Assuming a dermal absorption value of 1% for the concentrate and 10% for the spray solution, the levels of systemic operator exposure are as summarised in Table B.6.109.

Table B.6.109 Systemic operator exposure based on dosimetry results.

Minimum total systemic exposure *		Maximum total systemic exposure *		75 <sup>th</sup> percentile total systemic exposure *	
mg/kg bw/day	% of AOEL	mg/kg bw/day	% of AOEL	mg/kg bw/day	% of AOEL
No PPE (or normal work wear) – based on PDE values					
0.000444 (0.007719)	9% (154%)	0.072147 ( )	1443% ( )	0.040127 (0.044339)	803% (887%)
PPE used in study (see below) – based on ADE values					
0.000158 (0.001182)	3% (24%)	0.020097 ( )	402% ( )	0.006793 (0.008302)	136% (166%)
* assuming a dermal absorption for methomyl of 1% for the concentrate and 10% for the spray solution PPE used in study: Coveralls, gloves and face shield when handling the concentrate Coveralls during application Coveralls and gloves when handling contaminated surfaces AOEL evaluator's proposed systemic AOEL of 0.005 mg/kg bw/day ( ) values in brackets exclude those operators using closed cabs (subjects 3 and 7)					

On the basis of both the 75th percentile and maximum values for dermal and inhalation exposure summarised above (the maximum values may be more appropriate as the AOEL is set on an acute study), the supported use of 'Methomyl 20SL' on grapevines is considered to result in an unacceptable level of operator

exposure to methomyl (1.4x to 4.0x the systemic AOEL of 0.005 mg/kg bw/day proposed in this evaluation) when operators wear:

- Coveralls, gloves and face shield when handling the concentrate
- Coveralls during application
- Coveralls and gloves when handling contaminated surfaces

Although the data for individual test subjects (Table B.6.107) indicate that the levels of dermal and inhalation exposure during application may be significantly reduced by the use of tractors with closed cabs, as only two operators used such equipment (subjects 3 and 7) there is insufficient evidence to demonstrate that the use of 'Methomyl 20SL' will result in an acceptable risk to operators when working in a closed cab. Also, the low number of operators using equipment with closed cabs in this study indicates that the use of such equipment is not the norm in typical commercial vineyards and it would, therefore, be inappropriate to specify this form of engineering control for users of 'Methomyl 20SL'.

Old J., Gubert L. and Anderson I. (2002)

#### B.6.14.2 Bystander exposure

Bystander exposure to methomyl resulting from the supported use of 'Methomyl 20SL' on grapevines and field crops is likely to be of a short duration, is unlikely to be repeated, and is likely to be at a lower level than that affecting the sprayer operator considering the greater distance of a bystander from the application equipment and lack of direct contact with the formulation. Such exposure is likely to result primarily from spray drift as methomyl is only moderately volatile (vapour pressure =  $7.2 \times 10^{-4}$  Pa at 25 °C).

The notifier has submitted an estimate of bystander exposure based on drift data published the EPPO (Ganzelmeier *et al.* 1995). These data indicate that the highest levels of drift are likely to result from the supported use of 'Methomyl 20SL' on grapevines. In this situation, a level of drift equivalent to 0.3% of the applied dose was measured at 10 m from the application equipment. Assuming that 'Methomyl 20SL' is applied at the maximum supported dose of 0.45 kg a.s./ha, the notifier has calculated that a 70 kg bystander with an exposed skin surface area of 1 m<sup>2</sup> (and no contamination in the unexposed area) will have a dermal exposure of 0.001 mg/kg bw/day. The notifier has assumed that inhalation exposure will be negligible for a bystander.

The levels of bystander exposure resulting from the supported uses of 'Methomyl 20SL' on field crops and grapevines may also be estimated on the basis of direct measurements of simulated bystander exposure for boom sprayers and broadcast air-assisted sprayers in UK studies<sup>3,4</sup>.

In the field crop sprayer study, a single pass of the sprayer resulted in a mean potential dermal exposure of 0.1 ml of spray solution on a bystander positioned 8 m from the edge of the treatment area. A typical mean air concentration sampled in the breathing zone of the bystander was determined to be 0.02 ml of spray solution/m<sup>3</sup>.

Assuming a maximum concentration of 0.9 mg methomyl *per* ml of spray solution, no exposure reduction from clothing, 10% dermal absorption for the spray solution, a heavy work respiratory rate of 3.6 m<sup>3</sup>/h, 5 minutes exposure, 100% absorption and retention of potential inhalation exposure, and a body weight of 60 kg; estimated total systemic bystander exposure to methomyl is calculated to be 0.00024 mg/kg bw (equivalent to approximately 5% of the systemic AOEL of 0.005 mg/kg bw/day proposed in this evaluation) based on the simulated bystander exposure values.

In the broadcast air-assisted sprayer study, treatment of a 0.9 ha orchard using an application volume of 470 l/ha resulted in a mean potential dermal exposure of 3.7 ml of spray solution on a bystander positioned at 8 m from the edge of the treatment area. Mean potential inhalation exposure was 0.002 ml of spray solution.

Assuming a maximum concentration of 1.17 mg methomyl *per* ml of spray solution, no exposure reduction from clothing, 10% dermal absorption for the spray solution, 100% absorption and retention of potential inhalation exposure, and a body weight of 60 kg; estimated total systemic bystander exposure to methomyl is calculated to be 0.0072 mg/kg bw (equivalent to 1.4x the systemic AOEL of 0.005 mg/kg bw/day proposed in this evaluation) based on the simulated bystander exposure values.

Although these estimates are based on an acute exposure situation and are compared with an acute AOEL, this AOEL is considered an appropriate basis for assessing the risks associated with repeated exposures for operators. Therefore, these bystander exposure assessments are also relevant to situations of repeated bystander exposure (e.g. the exposure of residents in property adjoining sprayed crops).

On the basis of these estimates, the risk to bystanders resulting from the supported use of 'Methomyl 20SL' on field crops is considered to be acceptable whereas further information is required to demonstrate that the use of this product on grapes will result in an acceptable risk to bystanders.

#### **B.6.14.3 Worker exposure**

The notifier has submitted 2 dislodgeable foliar residue (DFR) decline studies (both GLP-compliant): one on lettuce foliage (as a surrogate for the field crops with low lying foliage on which 'Methomyl 20SL' is intended for use) and one on grape foliage.



### Field crops

The DFR study on lettuce was carried out in 1997 (field phase) at 2 commercial growers: one in Tulare County, California, USA and the other in Orange County, Florida, USA. Samples of lettuce leaves (25.4 mm diameter punched discs) were collected following 2 applications of 1.0 kg methomyl/ha (i.e. a total dose 2.0 kg methomyl/ha) with an interval of 2 days between applications. The products used were a SL formulation containing a measured concentration of 29.9% w/v methomyl and a SP formulation containing a measured concentration of 91.2% w/w methomyl, and both were applied using tractor-mounted/trailed field crop sprayers in a water volume of 487 to 497 l/ha.

Leaf samples (40 discs for each sampling) were taken 1 hour before the last application and following the last application at 1 hour, 12 hours, 1, 2, 4, 7, 14, 28 and 35 days. Samples were washed twice in 100 ml aliquots of a 0.01% v/v Aerosol OT 75 detergent solution to dislodge the foliar residues within 4 hours of collection. All samples were frozen during storage and transport and were analysed within 3 months of dislodging. Clean-up of combined dislodging solutions was achieved by the addition of saturated sodium chloride solution followed by partitioning with methylene chloride. Levels of methomyl were determined by HPLC/UV (see Section B.5.2.1). The LOQ for dislodged methomyl residues on lettuce was 0.001 µg/cm<sup>2</sup>. Field fortified samples, generated on days 1, 14, and 28 after the last treatment and handled, shipped, stored and analysed with the field specimens, demonstrated no losses. Average recovery data for laboratory fortifications run concurrently with treated specimens are presented in Table B.6.110.

Table B.6.110. Recovery data for dislodged methomyl residues on lettuce.

Fortification level (µg/cm <sup>2</sup> )	Number of samples	Percent recovery (mean ± S.D.)
0.005	2	92 ± 7
0.0125	4	96 ± 4
0.025	5	94 ± 6
0.125	7	91 ± 2
1.000	9	94 ± 1
Overall	27	94 ± 3

A summary of all foliage residue data is presented in Table B.6.112. The average amount of dislodged methomyl residues on lettuce foliage immediately before the last application was approximately 0.74 µg/cm<sup>2</sup> (range 0.392 to 1.094 µg/cm<sup>2</sup>). Foliar residues declined from 1.372 to 2.891 µg/cm<sup>2</sup> immediately after the last application to approximately ≤0.001 µg/cm<sup>2</sup> at the 35-day sampling interval. The foliar dissipation half-life of methomyl residues on lettuce foliage was 1.6 and 0.66 days for the California and Florida test sites, respectively.

The California site received a total rainfall of 102 mm during the test period (from the first application to the final sampling) which was approximately 1.5x the 10-year mean value for this period from data compiled at the nearest meteorological

station. Daily air temperatures recorded at this site over the study period ranged from 5 to 21 °C. The Florida site received a total rainfall of 481 mm during the test period (from the first application to the final sampling) which was approximately 3x the 10-year mean value for this period from data compiled at the nearest meteorological station (although this site was also irrigated, the foliage was not affected). Daily air temperatures recorded at this site over the study period ranged from 12 to 23 °C.

Although the methomyl formulations used in this study differ from 'Methomyl 20SL', the composition of the spray solutions are likely to be similar. However, the application rate used in the study (2 applications of 1.0 kg methomyl/ha) is higher than that specified for the supported use of 'Methomyl 20SL' on field crops (2 applications of 0.45 kg methomyl/ha) and the interval between applications used in the study (2 days) is lower than the 14 day interval specified for the supported use. Adjusting the highest DFR recorded in the study 7 DAT2 (the supported harvest interval for the use of 'Methomyl 20SL' on field crops) to reflect the difference between the application rate used in the study and that proposed for the use of 'Methomyl 20SL' gives a corrected DFR of  $6.75 \times 10^{-6}$  mg a.s./cm<sup>2</sup>. Assuming a transfer coefficient for workers harvesting cucumber, courgette, tomato and eggplant of 8000 cm<sup>2</sup>/h (a high value for 'reach and pick tasks' in similar crops based on published information<sup>5</sup>), 8 hours exposure, a dermal absorption of 10% for methomyl in transferred foliar residues and a 60 kg body weight, systemic exposure for unprotected workers is estimated to be 0.00072 mg/kg bw/day (approximately 14% of the systemic AOEL of 0.005 mg/kg bw/day proposed in this evaluation).

Although it is unclear whether any differences between the climatic conditions recorded in the DFR studies and those likely to be found in southern Europe are likely to effect significantly the decline of foliar residues, this is a worst case estimate based on a DFR resulting from an application interval of only 2 days (rather than the 14 day interval specified for the supported use).

On this basis, the risk to workers resulting from the supported use 'Methomyl 20SL' on field crops is considered to be acceptable.

Merricks, D.L. and Slaughter, C.J. (1998)

### Grapevines

The DFR study on grapevines was carried out in 1997 (field phase) at 2 commercial vineyards in Tulare County, California, USA. Samples of grape vine leaves (25.4 mm diameter punched discs) were collected following 2 applications of 1.0 kg methomyl/ha (i.e. a total dose 2.0 kg methomyl/ha) with an interval of 5 days between applications. The products used were a SL formulation containing a measured concentration of 29.9% w/v methomyl and a SP formulation containing a measured concentration of 91.2% w/w methomyl, and both were applied using tractor-mounted/trailed broadcast air-assisted sprayers in a water volume of 480 to 518 l/ha.

Leaf samples (40 discs for each sampling) were taken 1 hour before the last application and following the last application at 1 hour, 1, 2, 4, 7, 10, 14, 21, 28

and 35 days. Samples were washed twice in 100 ml aliquots of a 0.01% v/v Aerosol OT 75 detergent solution to dislodge the foliar residues within 4 hours of collection. All samples were frozen during storage and transport and were analysed within 3 months of dislodging. Clean-up of combined dislodging solutions was achieved by the addition of saturated sodium chloride solution followed by partitioning with methylene chloride. Levels of methomyl were determined by HPLC/UV (see Section B.5.2.1). The LOQ for dislodged methomyl residues on grapevines was 0.001 µg/cm<sup>2</sup>. Field fortified samples, generated on days 1, 14, and 28 after the last treatment and handled, shipped, stored and analysed with the field specimens, demonstrated no losses. Average recovery data for laboratory fortifications run concurrently with treated specimens are presented in Table B.6.111.

Table B.6.111. Recovery data for dislodged methomyl residues on grapevines.

Fortification level (µg/cm <sup>2</sup> )	Number of samples	Percent recovery (mean ± S.D.)
0.025	8	96 ± 5
0.125	8	94 ± 4
1.000	12	93 ± 5
Overall	28	94 ± 5

A summary of all foliage residue data is presented in Table B.6.113. The average amount of dislodged methomyl residues on grapevine foliage immediately before the last application was approximately 0.11 µg/cm<sup>2</sup> (range 0.047 to 0.173 µg/cm<sup>2</sup>). Foliar residues declined from 0.823 to 1.385 µg/cm<sup>2</sup> immediately after the last application to <0.001 µg/cm<sup>2</sup> at the 35-day sampling interval. The foliar dissipation half-life of methomyl residues on grapevine foliage was 3.6 and 3.0 days at the 2 Californian test sites.

The sites received a total rainfall of 1.3 mm during the test period (from the first application to the final sampling) which was approximately 9% of the 10-year mean value for this period from data compiled at the nearest meteorological station. Daily air temperatures recorded at these sites over the study period ranged from 11 to 36 °C.

Although the methomyl formulations used in this study differ from 'Methomyl 20SL', the composition of the spray solutions are likely to be similar. However, the application rate used in the study (2 applications of 1.0 kg methomyl/ha) is higher than that specified for the supported use of 'Methomyl 20SL' on grapevines (2 applications of 0.45 kg methomyl/ha) and the interval between applications used in the study (2 days) is shorter than the 14 day interval specified for the supported use. Adjusting the highest DFR recorded in the study 14 DAT2 (the supported harvest interval for the use of 'Methomyl 20SL' on grapes) to reflect the difference between the application rate used in the study and that proposed for the use of 'Methomyl 20SL' gives a corrected DFR of 3.6 x 10<sup>-6</sup> mg a.s./cm<sup>2</sup>. Assuming a transfer coefficient for workers harvesting grapes of 30000 cm<sup>2</sup>/h (a high value for 'search, reach and pick tasks' in similar crops based on published information<sup>5</sup>), 8 hours exposure, a dermal absorption of 10% for methomyl in transferred foliar

residues and a 60 kg body weight, systemic exposure for unprotected workers is estimated to be 0.00144 mg/kg bw/day (approximately 29% of the systemic AOEL of 0.005 mg/kg bw/day proposed in this evaluation).

Although it is unclear whether any differences between the climatic conditions recorded in the DFR studies and those likely to be found in southern Europe are likely to effect significantly the decline of foliar residues, this is a worst case estimate based on a DFR resulting from an application interval of only 2 days (rather than the 14 day interval specified for the supported use).

On this basis, the risk to workers resulting from the supported use 'Methomyl 20SL' on grapevines is considered to be acceptable.

Merricks, D.L. and McNeal, H.R. (1998)

Table B.6.112. Summary of mean dislodged residue from lettuce foliage.

Mean dislodged residue from lettuce foliage (residue in dislodging solution expressed as µg methomyl/cm <sup>2</sup> of lettuce foliage)															
Location	Application parameters			Sampling time (days after last application)											Half life days <sup>a</sup>
	Formul'n	No	kg a.s./ ha	-0.04	0.04	0.5	1	2	4	7	14	21	28	35	
California	29 % SL	2	1.0	0.392	1.372	0.995	0.250	0.479	0.112	0.015	0.003	0.002	0.001	0.001	1.6
Florida	90% SP	2	1.0	1.094	2.891	1.683	0.408	0.049	0.009	0.002	<0.001 (0.0008)	<0.001	<0.001 (0.0003)	-- <sup>b</sup>	0.66

<sup>a</sup> Half-life (first-order linear regression DT<sub>50</sub>) for each trial is shown in bold print.<sup>b</sup> No analysis performed due to <LOQ residues in previous two samplings.Table B.6.113. Summary of mean dislodged residue from grapevine foliage.

Mean dislodged residue from grapevine foliage (residue in dislodging solution expressed as µg methomyl/cm <sup>2</sup> of grapevine foliage)															
Location	Application parameters			Sampling time (days after last application)											Half life days <sup>a</sup>
	Formul'n	No	kg a.s./ ha	-0.04	0.04	1	2	4	7	10	14	21	28	35	
California 1	29 % SL	2	1.0	0.047	0.823	0.486	0.302	0.078	0.033	0.029	0.008	0.002	0.002	<0.001 (0.0009)	3.6
California 2	90% SP	2	1.0	0.173	1.385	0.962	0.609	0.177	0.142	0.026	0.007	0.011	0.002	<0.001 (0.0003)	3.0

<sup>a</sup> Half-life (first-order linear regression DT<sub>50</sub>) for each trial is shown in bold print.

#### B.6.14.4 Summary of risk to operators, bystanders and workers

Operator exposure estimates using the German model indicate that the use of 'Methomyl 20SL' on grapes through tractor-mounted/trailed equipment will result in an unacceptable level of systemic exposure to methomyl for an operator wearing protective gloves when handling the concentrate and coveralls and protective gloves during application (systemic exposure equivalent to 1.9x the systemic AOEL of 0.005 mg/kg bw/day proposed in this evaluation). The corresponding UK POEM estimates also indicate an unacceptable level of systemic exposure for an operator wearing protective gloves when handling the concentrate and during application (systemic exposure equivalent to 30x to 34x the proposed systemic AOEL). Similarly, calculations based on operator monitoring (dosimetry) data indicate that the supported use of 'Methomyl 20SL' on grapevines will result in an unacceptable level of exposure to methomyl (up to 4x the systemic AOEL of 0.005 mg/kg bw/day proposed in this evaluation) when operators wear coveralls, gloves and face shield when handling the concentrate; coveralls during application; and coveralls and gloves when handling contaminated surfaces.

Operator exposure estimates using the German model indicate that the use of 'Methomyl 20SL' on field crops through tractor-mounted/trailed equipment will result in an acceptable level of systemic exposure to methomyl for a operator wearing protective gloves when handling the concentrate and coveralls and protective gloves during application (systemic exposure equivalent to 21% to 42% of the systemic AOEL of 0.005 mg/kg bw/day proposed in this evaluation). The corresponding UK POEM estimates indicate an unacceptable level of systemic exposure for an operator wearing protective gloves when handling the concentrate and when handling contaminated surfaces (systemic exposure equivalent to 22x to 25x the proposed systemic AOEL).

Although the use of 'Methomyl 20SL' through hand-held sprayers on field crops and grapes is not being supported by the notifier, operator exposure estimates indicate that such uses may result in an unacceptable risk to operators.

Bystander exposure estimates based on published field study measurements indicate that the level of systemic exposure to methomyl for an unprotected bystander at the time of application is likely to be acceptable for field crops but unacceptable for grapes (equivalent to 5% of the systemic AOEL of 0.005 mg/kg bw/day and 1.4x the systemic AOEL, respectively).

Worker exposure estimates based on dislodgeable foliar residue decline studies and using published transfer coefficient data indicate that the levels of systemic exposure to methomyl for an unprotected worker harvesting treated field crops and grapes are likely to be acceptable (equivalent to 14% and 29% of the systemic AOEL of 0.005 mg/kg bw/day, respectively).

**B.6.15 References relied on**

Author(s)	Annex No., Reference No.	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	EU Data Protection Claimed (Y/N)	Owner
Anon	various	2004	Du Pont Comments on methomyl first version of the Draft Assessment Report 19 March 2004		DuPont
Anon.	IIA, 5.4.2./01	1984	<i>In vivo</i> bone marrow chromosome study in rats Hazleton Laboratories America, Inc., 9200 Leesburg Turnpike HLO 63-84 GLP: No Published: No	Y	DuPont
Arondi, S.	IIA, 5.2.6./01	1991	Closed-patch repeated insult dermal sensitization study (Buehler method) with DPX-X1179-394 in Guinea pigs Pharmakon Research International, Inc., P.O. Box 313 HLO 345-91, Revision 1 GLP: Yes Published: No	Y	DuPont
Bentley, K.S.	IIA, 5.4.2./02	1995	Mouse bone marrow micronucleus assay of DPX-X1179-394 DuPont Haskell Laboratory, P.O. Box 50, Elkton Road HLR 413-95 GLP: Yes Published: No	Y	DuPont
Brock, W.J.	IIA, 5.3.3./01	1989	Repeated dose dermal toxicity: 21-day study with DPX-X1179-394 (methomyl) in rabbits DuPont Haskell Laboratory, P.O. Box 50, Elkton Road HLR 387-89 GLP: Yes Published: No	Y	DuPont
Busey, W.M.	IIA, 5.3.2./01	1966	Three month dietary administration - rats insecticide 1179 Hazleton Laboratories America, Inc., 9200 Leesburg Turnpike Hazleton 1466 GLP: No Published: No	Y	DuPont
Busey, W.M.	IIA, 5.5.5./01	1968	Two-year dietary administration - dogs Lannate <sup>®</sup> methomyl insecticide (S-methyl-N-((methylcarbamoyl)oxy) thioacetimidate) Hazleton Laboratories America, Inc., 9200 Leesburg Turnpike Hazleton 201-165 and accompanying Addendum No. 1 GLP: No Published: No	Y	DuPont

Author(s)	Annex No., Reference No.	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	EU Data Protection Claimed (Y/N)	Owner
Carakostas, M.C.	IIA, 5.8.2./08	1987	Inhibition and regeneration kinetics for human and rat acetylcholinesterase exposed to methomyl - <i>in vitro</i> DuPont Haskell Laboratory, P.O. Box 50, Elkton Road HLR 379-88 GLP: No Published: No	Y	DuPont
Culik, R., Rogers, A.S.	IIA, 5.6.2./01	1978	Oral teratogenic study in rats with Lannate® (INX-1179) DuPont Haskell Laboratory, P.O. Box 50, Elkton Road HLR 498-78 GLP: No Published: No	Y	DuPont
Fasano, W.J.	IIA, 5.8.2./10	2001	Methomyl (DPX-X1179) 20SL: <i>in vivo</i> dermal absorption of [1- <sup>14</sup> C] methomyl in the rat DuPont Haskell Laboratory DuPont-5697 GLP: Yes Published: No	Y	DuPont
Fasano, W.J.	IIA, 5.8.2./09	2001	Methomyl: <i>in vitro</i> dermal kinetics of [1- <sup>14</sup> C]methomyl in rat, human, and rabbit skin (Lannate® 20L formulation) DuPont Haskell Laboratory DuPont-5835 GLP: Yes Published: No	Y	DuPont
Feussner, E.L., Hoberman, A.M., Christian, M.S.	IIA, 5.6.2./02	1983	Embryo-fetal toxicity and teratogenicity study of methomyl in the rabbit Argus Research Laboratories, Inc., 935 Horsham Road HLO 331-83 GLP: No Published: No	Y	DuPont
Filliben, T.	IIA, 5.8.2./06	1996	Acute dietary toxicity study for cholinesterase inhibition with DPX-X1179 in male rats DuPont Haskell Laboratory, P.O. Box 50, Elkton Road HLR 861-96 GLP: Yes Published: No	Y	DuPont
Finlay, C.	IIA, 5.3.3./02	1997	Methomyl technical: 21-day repeated dose dermal toxicity study in rabbits DuPont Haskell Laboratory, P.O. Box 50, Elkton Road HL-1997-00913 GLP: Yes Published: No	Y	DuPont



Author(s)	Annex No., Reference No.	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	EU Data Protection Claimed (Y/N)	Owner
Harvey, J. Jr., Buchanan, J.B.	IIA, 5.1.2./02	1968	Absence of S-oxide and S,S dioxide as potential metabolites of methomyl in soil, tobacco and rats DuPont Experimental Station ML/ME 25 GLP: No Published: No	Y	DuPont
Harvey, J. Jr., Jelinek, A.G., Sherman, H.	IIA, 5.1.2./01	1967	Metabolism of S-methyl N-[(methylcarbamoyl)oxy]thioacetimidate in the rat DuPont Experimental Station ML/ME 24 GLP: No Published: No	Y	DuPont
Harvey, J. Jr., Jelinek, A.G., Sherman, H.	IIA, 5.1.2./03	1973	Metabolism of methomyl in the rat DuPont Experimental Station ML/ME 50 GLP: no Published: Yes	N	Authors
Hawkins, D.R., Mayo, B.C., Pollard, A.D., Haynes, L.M.	IIA, 5.1.1./01	1991	The metabolism of [1- <sup>14</sup> C] methomyl in rats Huntingdon Research Centre (UK) AMR 1584-90 GLP: Yes Published: No	Y	DuPont
Hawkins, D.R., Mayo, B.C., Pollard, A.D., Haynes, L.M.	IIA, 5.1.3./01	1992	The metabolism of [1- <sup>14</sup> C] methomyl in male Cynomolgus monkey Huntingdon Research Centre (UK) AMR 1902-90 GLP: Yes Published: No	Y	DuPont
Huhtanen, K., Dorough, H. W.	IIA, 5.1.1./02	1976	Isomerization and Beckman Rearrangement Reactions in the Metabolism of Methomyl in Rats Pesticide Biochem Physiol Vol. 6 GLP: No Published: Yes	N	Pesticide Biochem Physiol
Kaplan, A.M.	IIA, 5.5.1./01	1981	Long-term feeding study in rats with S-methyl N-((methylcarbamoyl)oxy)thioacetimidate (methomyl; INX-1179) DuPont Haskell Laboratory, P.O. Box 50, Elkton Road HLR 235-81, 5 volumes GLP: No Published: No	Y	DuPont
Malley, L.A.	IIA, 5.8.2./04	1997	Reversibility study with carbamate insecticides in rats DuPont Haskell Laboratory, P.O. Box 50, Elkton Road HL-1997-00641 GLP: Yes Published: No	Y	DuPont

Author(s)	Annex No., Reference No.	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	EU Data Protection Claimed (Y/N)	Owner
Mathison, B.H.	IIA, 5.4.1./01	1997	DPX-X1179 (methomyl): Mutagenicity testing in the <i>Salmonella typhimurium</i> and <i>Escherichia coli</i> plate incorporation assay DuPont Haskell Laboratory, P.O. Box 50, Elkton Road HL-1997-00043 GLP: Yes Published: No	Y	DuPont
McCooley, K.T.	IIA, 5.4.1./02	1984	CHO/HGPRT assay for gene mutation DuPont Haskell Laboratory, P.O. Box 50, Elkton Road HLR 556-83, Revision 1 GLP: No Published: No	Y	DuPont
McFarlane, P., Sanderson, J.B., Freestone, S.	IIA, 5.8.2./03	1998	A randomised double blind ascending oral dose study with methomyl to establish a no adverse effect level Inveresk Research International (IRI) Limited, Research Park (Scotland) HLO-1998-00969 (2 volumes) GLP: Yes Published: No	Y	DuPont
Merricks, D.L., McNeal, H.R.	IIA, 6.3.6.2./01	1998	Dissipation of dislodgeable foliar and soil residues of methomyl from grapes following application of Lannate□ LV or Lannate□ SP insecticide in the U.S.A. - season 1997 Agrisearch, Inc. AMR 4449-97 GLP: Yes Published: No	Y	DuPont
Merricks, D.L., Slaughter, C.J.	IIA, 6.3.6.1./01	1998	Dissipation of dislodgeable foliar residues of methomyl from lettuce following application of Lannate□ LV insecticide or Lannate□ SP insecticide in the U.S.A. - season 1997 Agrisearch, Inc. AMR 4450-97 GLP: Yes Published: No	Y	DuPont
Mikles, K. A.	IIA, 5.8.2./01	1998	Methomyl technical (DPX-X1179-512): Acute oral neurotoxicity study in rats DuPont Haskell Laboratory, P.O. Box 50, Elkton Road HL-1998-01080 (2 volumes) GLP: Yes Published: No	Y	DuPont

Author(s)	Annex No., Reference No.	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	EU Data Protection Claimed (Y/N)	Owner
Mikles, K.A.	IIA, 5.8.2./02	1998	Methomyl technical (DPX-X1179-512): subchronic oral neurotoxicity study in rats DuPont Haskell Laboratory, P.O. Box 50, Elkton Road HL-1998-01639 (2 volumes) GLP: Yes Published: No	Y	DuPont
Panepinto, A.S.	IIA, 5.2.3./01	1991	Acute inhalation toxicity study with DPX-X1179-427 in rats DuPont Haskell Laboratory, P.O. Box 50, Elkton Road HLR 678-91 GLP: Yes Published: No	Y	DuPont
Sarver, J.W.	IIA, 5.2.1./01	1991a	Acute oral toxicity study with DPX-X1179-394 in male and female rats DuPont Haskell Laboratory, P.O. Box 50, Elkton Road HLR 661-91 GLP: Yes Published: No	Y	DuPont
Sarver, J.W.	IIA, 5.2.2./01	1991b	Acute dermal toxicity study with DPX-X1179-394 in rabbits DuPont Haskell Laboratory, P.O. Box 50, Elkton Road HLR 455-91 GLP: Yes Published: No	Y	DuPont
Sarver, J.W.	IIA, 5.2.4./01	1993	Primary dermal irritation study with DPX-X1179-394 in rabbits DuPont Haskell Laboratory, P.O. Box 50, Elkton Road HLR 563-93 GLP: Yes Published: No	Y	DuPont
Sarver, J.W.	IIA, 5.2.5./01	1991c	Primary eye irritation study with DPX-X1179-425 in rabbits DuPont Haskell Laboratory, P.O. Box 50, Elkton Road HLR 280-91 GLP: Yes Published: No	Y	DuPont

Author(s)	Annex No., Reference No.	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	EU Data Protection Claimed (Y/N)	Owner
Scott, D.G.	IIA, 5.8.2/04	1998	Validation of analytical method for methomyl capsules and the use of this method for the determination of dissolution rate and formulation accuracy Inveresk Research International (IRI), Limited, Research Park (Scotland) HLO-1998-00974 GLP: Yes Published: No	Y	DuPont
Vincent, D.R.	IIA, 5.4.1./03	1985	Assessment of methomyl (INX-1179-255) in the <i>in vitro</i> unscheduled DNA synthesis assay in primary rat hepatocytes DuPont Haskell Laboratory, P.O. Box 50, Elkton Road HLR 149-85, Revision 1 GLP: No Published: No	Y	DuPont

### Plant protection product – Methomyl 20 SL

Author(s)	Annex No., Reference No.	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	EU Data Protection Claimed (Y/N)	Owner
Finlay, C.	IIIA, 7.1.1./01	1999	Methomyl 20L: acute oral toxicity study in male and female rats DuPont Haskell Laboratory DuPont-3282 GLP: Yes Published: No	N	DuPont
Finlay, C.	IIIA, 7.1.2./01	1999	Methomyl 20L: acute dermal toxicity study in rats DuPont Haskell Laboratory DuPont-3193 GLP: No Published: Yes	N	DuPont
Finlay, C.	IIIA, 7.1.5./01	1999	Methomyl 20L: primary eye irritation study in rabbits DuPont Haskell Laboratory DuPont-3286 GLP: Yes Published: No	N	DuPont

Author(s)	Annex No., Reference No.	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	EU Data Protec- tion Claimed (Y/N)	Owner
Finlay, C.	IIIA, 7.1.4./01	1999	Methomyl 20L: primary dermal irritation study in rabbits DuPont Haskell Laboratory DuPont-3158 GLP: Yes Published: No	N	DuPont
Hershman, R.J.	IIIA, 7.1.6./01	1999	Methomyl 20L: evaluation of the potential dermal sensitization in the guinea pig (Modified Buehler Method) White Eagle Toxicology Laboratories DuPont-3022 GLP: Yes Published: No	N	DuPont
Jackson, G.C., Hardy, C.J., Gregson, R.L., Offer, J.M., Gopinath, C.	IIIA, 7.1.3./01	1991	Lannate <sup>®</sup> 20L acute inhalation toxicity in rats 4-hour exposure Huntingdon Research Centre DPT 247/91521 GLP: No Published: No	N	DuPont
Old, J., Gubert, L., Anderson, I.	IIIA, 7.2.1.2./01	2002	Determination of dermal and inhalation exposure of operators during mixing/loading and application using Methomyl 20SL, a soluble concentrate containing methomyl 200 g/L applied via mist applicators in grapes Inveresk Research (Scotland) DuPont-6771 GLP: Yes Published: No	N	DuPont
Salvi, M.	IIIA, 7.2.1.2./02	2002	Validation of an analytical method for the determination of methomyl in specimens before operator exposure study ADME BIOANALYSES (France) DuPont-6706 GLP: Yes Published: No	N	DuPont

### **References used in assessment of data (mammalian toxicology)**

Buchholz, U, Mermin, J, Rios, R, Casagrande, T L, Galey, F, Lee, M, Quattrone, A, Farrar, J, Nagelkirke, N, and Werner, S B (2002). *An outbreak of food-borne illness associated with methomyl-contaminated salt* J. American Medical Association 288, 5, 604-610.

**References used in assessment of data (operator exposure)**

1. BBA (1992). Uniform Principles for Safeguarding the Health of Applicators of Plant Protection Products. Biologische Bundesanstalt für Land- und Forstwirtschaft, Bundesgesundheitsamt, und Industrieverband Agrar e.V. ISBN 3489-27700-7.
2. PSD (1986). UK Scientific Subcommittee on Pesticides & British Agrochemicals Association, Joint Medical Panel; UK Predictive Operator Exposure Model (POEM): Estimation of Exposure and Absorption of Pesticides by Spray Operators.
3. Lloyd G.A. and Bell G.J. (1983). Hydraulic nozzles: comparative spray drift study (SC7704).
4. Lloyd G.A., Cross J.V. et al, 1987. Orchard sprayers: comparative operator exposure and spray drift study (MAFF/ADAS).
5. Krieger R.I., Ross J.H. and Thongsinthusak T. (1992). Assessing Human Exposures to Pesticides. Rev. Env. Contam. Tox. 128 pp. 1-15.

WARNING: This document forms part of an EC evaluation data package and should not be read in isolation. Registration must not be based on the basis of this document.

**B.7 Residues data****B.7.1 Metabolism, distribution and expression of residues in plants (IIA 6.1, IIIA 8.1)****B.7.1.1 Grapevine**

The metabolism and distribution of methomyl was investigated using [ $1\text{-}^{14}\text{C}$ ] –labelled methomyl (radiochemical purity >96%) in a 2001 study. A test solution of a mix of radiolabelled and non-labelled methomyl in a soluble concentrate formulation was applied as a foliar spray to grapevine (cv. Fredonia, approximately 32 years old) grown in an outdoor site in Delaware, USA. One application at a rate of 990g ai/ha was applied equivalent to a 2.2 N individual dose (1.1N maximum total dose). Grape berry and foliage samples were taken immediately after application and at 2, 7 and 14 days after application.

Bunches of grapes were initially washed with methanol: water (95:5 v/v) and the rinsate collected for analysis. The stems were removed and the grapes were homogenised with dry ice. Grape samples were extracted with methanol, followed by two successive extractions with methanol: water (95:5 v/v). The non-extracted residue was successively subjected to cellulase digestion, sonication with mild acid (0.1N hydrochloric acid), reflux with 6N hydrochloric acid and reflux with 4N sodium hydroxide. An aliquot of the combined berry methanol extracts from the Day 14 samples was subjected to enzyme hydrolysis with  $\beta$ -glucosidase.

Grape foliage was placed in plastic bags immediately after sampling. The foliage was removed and homogenised with dry ice. Any condensation present in the sample bags was collected. The bags were further rinsed with water and the rinsings added to the collected condensation. Foliage samples were extracted with three successive aliquots of methanol: water (70:30 v/v) and the extracts combined.

Radioactivity was analysed by LSC or LSC following combustion. Characterisation of the radioactive residues was performed using HPLC with a photodiode array detector against reference standards. Further characterisation was performed using LC-MS.

Levels of radioactivity detected in the samples are shown in tables B.7.1 and B.7.2. TRR were 1.282 mg/kg in grapes immediately after application falling to 0.942 mg/kg 14 days after. For grapevine leaves TRR were initially 48.6 mg/kg and 21.2 mg/kg 14 days after application. The majority of TRR were extracted in both commodities (70 - 100%).

Table B.7.1 Radioactive residues in grape berries treated with [ $^{14}\text{C}$ ] - labelled methomyl

	Day							
	0		2		7		14	
FRACTION	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
Berry Rinse	74.9	0.961	5.2	0.064	1.2	0.014	9.5	0.005
Combined Methanol: Water Extracts	24.8	0.318	90.9	1.123	91.1	1.039	87.7	0.828
Total Extractable	99.7	1.279	96.1	1.188	92.4	1.053	88.2	0.833
Unextracted Radioactivity	0.3	0.004	3.9	0.048	7.6 <sup>a</sup>	0.087 <sup>a</sup>	11.8 <sup>a</sup>	0.111 <sup>a</sup>
<b>Exhaustive Extraction of Unextracted Residues</b>								
Cellulase	-	-	-	-	1.0	0.012	1.4	0.013
0.1N hydrochloric acid	-	-	-	-	0.7	0.008	1.1	0.010
6N hydrochloric acid	-	-	-	-	2.3	0.027	2.6	0.025
4N sodium hydroxide	-	-	-	-	0.5	0.017	1.7	0.016
Bound Residues	-	-	-	-	2.8	0.032	4.7	0.045
Total Recovered Radioactivity <sup>b</sup>	100.0	1.282	100.0	1.235	100.8	1.149	99.8	0.942

a Day 7 and Day 14 unextracted radioactivity was released using exhaustive extraction techniques.

b Total recovered radioactivity is the sum of the berry rinse, extract, and initially unextracted residue except for Day 7 and Day 14 data, which reflect recovery from exhaustive extraction techniques.

Table B.7.2 Radioactive residues in grapevine foliage treated with [ $^{14}\text{C}$ ] - labelled methomyl

	Day							
	0		2		7		14	
FRACTION	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
Sample Bag Rinse <sup>a</sup>	5.9	2.9	0.58	0.3	0.1	<0.02	<0.1	<0.02
Combined Methanol :Water Extracts	92.7	45.0	90.4	47.1	72.4	13.7	68.1	14.4
Total Extractable	98.6	47.9	91.0	47.4	72.5	13.7	68.1	14.4
Unextracted Radioactivity	1.4	0.7	9.0	4.7	27.5	5.2	31.9	6.8
Total Recovered Radioactivity <sup>b</sup>	100.0	48.6	100.0	52.1	100.0	18.9	100.0	21.2

a radioactivity recovered from sample bag condensate and rinse

b Total recovered radioactivity is the sum of the sample bag rinse, extract and unextracted residue.

For grape berries over 90% TRR were characterised. The major metabolite identified was the parent methomyl accounting for 82.6% TRR (1.021 mg/kg) in samples harvested 2 days after application and 51.4% TRR (0.485 mg/kg) 14 days after application. For the 14 day samples other significant metabolites identified were methomyl oxime (IN-X1177) (7.2% TRR, 0.068 mg/kg) and IN-HUZ57 (5.6% TRR, 0.052 mg/kg). Approximately 11% TRR (0.102 mg/kg) were attributed to an unidentified polar fraction. This fraction was found to consist of at least 7 components



none of which were found at levels greater than 3.1% TRR (0.03 mg/kg). Radioactive components that co-eluted with glucose and fructose were also found in this fraction again at levels less than 3.1% TRR (0.03 mg/kg). No other metabolites were found in significant levels in the samples harvested 14 days after application. Table B.7.3 shows the levels of metabolites found in grape berry samples.

Grape foliage samples were extracted and analysed to further elucidate the metabolic pathway in grapevine. Over 68% of TRR were characterised. The major metabolite identified in samples harvested immediately after application was the parent methomyl accounting for 96.7% TRR (46.97 mg/kg). Methomyl accounted for 3.6% TRR (0.763 mg/kg) 14 days after application. The major identified metabolite in the day 14 samples was IN-HUZ57 accounting for 16.4% TRR (3.468 mg/kg). No further characterisation work on the un-extracted residue was performed. The proposed metabolic pathway of methomyl in grapevines is shown in Figure B.7.1.

(Ryan, D.L., McMillan, J.A., Young, G.A., 2003)

Table B.7.3 Characterisation of radioactive residues in grape berries treated with [ $^{14}\text{C}$ ] - labelled methomyl

Compound	Sample							
	Day 0		Day 2		Day 7		Day 14 <sup>a</sup>	
	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
Polar Fraction(s)	0.2	0.003	2.5	0.031	7.0	0.080	10.8 <sup>b</sup>	0.102 <sup>b</sup>
Acetic Acid	<0.1	<0.002	<0.1	<0.002	1.1	0.012	3.4	0.032
Acetonitrile (IN-07467)	<0.1	<0.002	3.4	0.013	3.4	0.039	3.8	0.036
IN-HUZ57	<0.1	<0.002	1.7	0.021	4.2	0.018	5.6	0.052
IN-G6250	0.3	0.004	0.1	0.001	0.8	0.010	1.2	0.012
Methomyl oxime (IN-X1177)	0.2	0.002	2.3	0.029	4.9	0.056	7.2	0.068
<b>Methomyl</b>	<b>96.9</b>	<b>1.243</b>	<b>82.6</b>	<b>1.021</b>	<b>64.5</b>	<b>0.735</b>	<b>51.4</b>	<b>0.485</b>
IN-B1871	0.3	0.003	0.1	0.001	0.6	0.007	0.7	0.007
IN-B1884	0.5	0.006	0.8	0.010	1.3	0.015	0.5	0.005
Unidentified Radioactivity >L <sub>D</sub> <sup>c</sup>	0.3	0.004	0.2	0.003	2.6	0.030	5.5	0.051
Unidentified Radioactivity >L <sub>C</sub> <L <sub>D</sub> <sup>d</sup>	1.1	0.014	2.3	0.029	1.9	0.022	3.3	0.031
Total %TRR Characterised by HPLC	98.6	1.265	93.8	1.159	90.4	1.031	90.0	0.849

a 14-day sample also includes data from cellulase, 0.1 N hydrochloric acid and 6 N hydrochloric acid extracts.

b 14-day polar fraction (methanol: water extract, 8.9% TRR, was found to consist of at least 7 components, none of which were greater than 3.1% TRR including radioactivity co-eluting with glucose/fructose (3.1% TRR) and near sucrose (2.1% TRR), all other components were individually less than 2% TRR. Acetamide (IN-09066, 0.7% TRR) was also part of this fraction.

c Five or more minor components detected, none of which were greater than 2.2% TRR.

d Radioactivity detected above L<sub>C</sub>, but below L<sub>D</sub>.

L<sub>C</sub> = critical limit = radioactivity that is differentiable from background but is not reliably detected.

L<sub>D</sub> = detection limit = radioactivity that is reliably detected; the L<sub>D</sub> for HPLC analyses was ~0.1% TRR for 14-day berry rinse and ~0.3% TRR for 14-day berry extract.

Table B.7.4 Characterisation of radioactive residues in grape foliage treated with [ $^{14}\text{C}$ ] - labelled methomyl

Compound	Sample							
	Day 0		Day 2		Day 7		Day 14 <sup>a</sup>	
	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
Methomyl oxime glucose conjugate and IN-HHC78	0.2	0.105	2.6	1.355	10.6	2.001	12.5 <sup>b</sup>	2.655 <sup>b</sup>
IN-HUZ57 glucose conjugate	<0.1	<0.002	0.9	0.447	6.0	1.138	6.1	1.294
IN-G6250 glucose conjugate	<0.1	<0.002	4.3	2.219	6.8	1.277	3.9	0.823
IN-NR282 glucose conjugate	<0.1	<0.002	0.9	0.477	4.4	0.824	8.1	1.708
IN-HUZ57	<0.1	0.002	8.1	4.218	20.7	3.914	16.4	3.468
IN-G6520	0.3	0.141	1.3	0.678	1.4	0.270	1.2	0.56
<b>Methomyl</b>	<b>96.7</b>	<b>46.966</b>	<b>66.7</b>	<b>34.784</b>	<b>9.0</b>	<b>1.705</b>	<b>3.6</b>	<b>0.763</b>
Unidentified Radioactivity >L <sub>D</sub> <sup>c</sup>	1.1	0.551	6.3	3.268	13.0	2.549	16.3	3.455
Unidentified	0.3	0.145	0.0	0.007	0.0	<0.002	0.0	0.002
Radioactivity >L <sub>C</sub> <L <sub>D</sub>								
Total %TRR characterised by HPLC	98.3	47.765	91.0	47.436	72.4	13.678	68.1	14.422

a 0-day and 2-day samples includes data sample bag rinse.

b Fraction (14-day extract, 12.5% TRR,) was found to consist of 2 major components in a 3:2 ratio methomyl oxime-glucose conjugate (7.5% TRR) and IN-HHC78 (5.0% TRR), unresolved in 0-, 2-, and 7-day extracts and reported as methomyl oxime glucose conjugate.

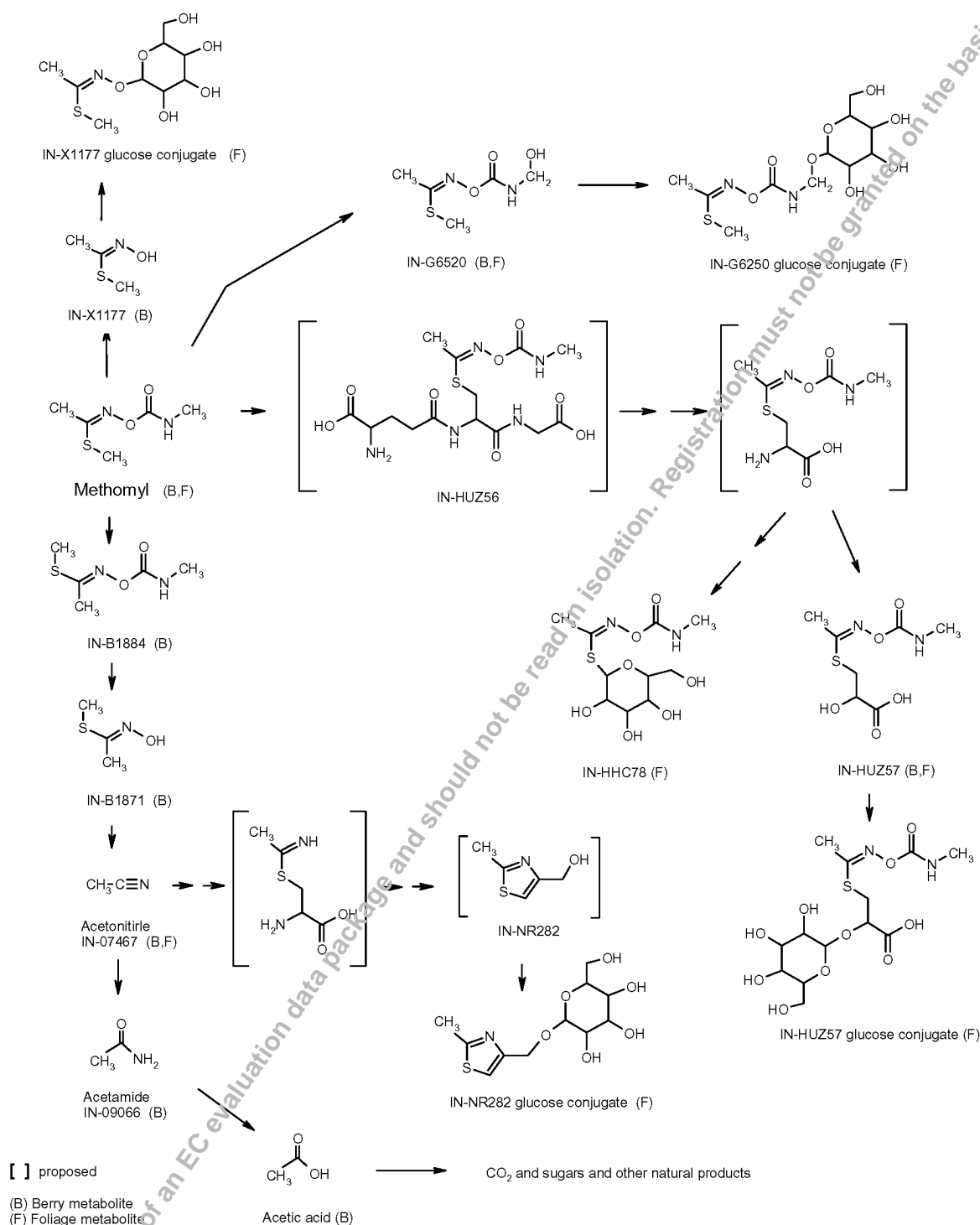
c Numerous minor components, no individual component greater than 3% TRR.

L = critical limit = radioactivity that is differentiable from background but is not reliably detected.

L<sub>C</sub> = detection limit = radioactivity that is reliably detected; the L<sub>D</sub> for HPLC analyses was ~0.1% TRR for 14 foliage-day extract

D

Figure B.7.1 Proposed metabolic pathway for methomyl in grapevine



### B.7.1.2 Tobacco

The metabolism of methomyl was investigated using [1-<sup>14</sup>C] -labelled methomyl (radiochemical purity >99 %\*) in studies performed in 1967 and 1968. In the first study the metabolism of methomyl in tobacco was investigated following absorption of methomyl via root treatment, and in the second study after foliar treatment.

Three young tobacco plants (variety Xanthi) were planted in quartz sand and enclosed in glass chambers designed to isolate the air space surrounding the aerial and the root portions of the plants from each other. The chambers were initially connected to a series of two sodium hydroxide traps designed to trap acidic volatile compounds such as  $^{14}\text{C}$ -carbon dioxide. A third sodium hydroxide trap was placed after an oxidising furnace in order to trap substances that had been oxidised to  $\text{CO}_2$ . The tobacco plants were treated with 1L of solution of  $10\text{ }\mu\text{g/ml}$  [ $1\text{-}^{14}\text{C}$ ]-methomyl which was re-circulated through the sand every second day. The plants were maintained with a nutrient solution. After four weeks the distribution of radioactivity was determined in two plants. The traps for the third plant were modified to consist of one sodium hydroxide trap, one dilute sulphuric acid trap, two cold traps immersed in dry ice-triclene and an oxidising furnace followed by a sodium hydroxide trap. This plant and trapping system was maintained for a further two weeks.

An additional experiment was conducted in which a single tobacco plant was treated with a solution of  $10\text{ }\mu\text{g/ml}$  [ $1\text{-}^{14}\text{C}$ ]-methomyl as described above. An extra cold trap was connected to the chamber preceding the sodium hydroxide traps and furnace in order to trap neutral volatile compounds for characterisation and identification.

Plant tissues (leaves, stems and roots) were pulverised and extracted three times with ethyl acetate. Radioactivity in plant tissue extracts, nutrition solution, volatile traps and condensate in the metabolism chamber were determined by LSC. The un-extracted radioactivity from plant tissue and radioactivity in the quartz sand were determined by LSC following combustion.

The ethyl acetate extracts from plant tissues were mixed with water and concentrated to a single aqueous fraction. The non-aqueous deposit was dissolved in benzene. Separation of metabolite fractions was performed using a counter-current fractionator. Radioactivity in the fractions was analysed by LSC and characterisation performed by TLC. Identification of methomyl was performed by Time of Flight-MS.

The radioactivity in the volatile traps was examined by precipitation with barium chloride and the filtered solution analysed by LSC. The radioactivity in the dry ice-triclene traps was partitioned between organic and aqueous phases and analysed by LSC and GC.

A further set of experiments were performed with a foliar treatment of two tobacco plants. The plants were covered with plastic wrap, leaving only the fifth leaf from the soil surface exposed. This leaf was treated with  $0.5\text{ ml}$  of a  $1000\text{ }\mu\text{g/ml}$  solution of [ $1\text{-}^{14}\text{C}$ ]-methomyl. After treatment, the plants were placed in a greenhouse. Samples of plant tissues were taken three and seven days after the treatment, extracted with ethyl acetate and analysed by LSC.

Table B.7.5 Distribution of [<sup>14</sup>C] - labelled methomyl equivalents in tobacco plant after root application (4 weeks)

Component	Plant 1		Plant 2	
	µCi	% dose <sup>a</sup>	µCi	% dose <sup>a</sup>
<b>Plant Tissues</b>				
Leaf extract	2.31	4.3	1.84	3.4
Leaf residue	0.8	1.5	0.78	1.4
Stem extract	0.08	0.1	0.08	0.1
Stem residue	0.08	0.1	0.09	0.2
Root extract	0.02	<0.1	0.03	<0.1
Root residue	0.04	<0.1	0.05	<0.1
<b>Volatile components</b>				
<sup>14</sup> C-Carbon dioxide <sup>b</sup>	4.67	8.6	3.62	6.7
[1- <sup>14</sup> C]Acetonitrile <sup>b</sup>	5.69	10.5	3.93	7.3
<b>Condensate <sup>c</sup></b>	0.09	0.2	0.29	0.5
Applied radioactivity absorbed by plants	13.78	25.4	16.71	19.7
<b>Growth media</b>				
Nutrient solution	37.92	70.0	41.67	77.0
Sand	0.3	0.6	0.35	0.7
<b>Total Recovery</b>	52.00	96.0	52.73	97.4

<sup>a</sup> Tobacco plants were treated with 54.2 µCi [1-<sup>14</sup>C]methomyl<sup>b</sup> Volatiles from aerial and root portions combined (all traps combined)<sup>c</sup> Condensed water in metabolism chamberTable B.7.6 Composition of tobacco leaf extracts after root application of [<sup>14</sup>C] - labelled methomyl (4 weeks)

Counter current Fraction	Fraction number	µCi <sup>a</sup>	% Fraction <sup>b</sup>	% Applied radioactivity <sup>c</sup>
Polar fraction	0-6	0.14	7.5	0.3
	10-25	<0.003	0.1	-
Methomyl	30-60	1.53	82.2	2.8
Non-polar fraction	80-94	0.19	10.2	0.4
<b>Total Residues:</b>		<b>1.86</b>	<b>100</b>	<b>3.5</b>

<sup>a</sup> µCi recovered in each fraction<sup>b</sup> % of extract found in these counter current fractions<sup>c</sup> % of total radioactivity applied to tobacco plant (54.2µCi total)

Table B.7.5 shows the distribution of radioactivity in the plants treated by root application. The plants absorbed 20-25% of the total radioactivity applied over a four-week period. The remainder of the radioactivity was found in the nutrition solution as an unchanged methomyl (70-77%). 5.2-6.1% was retained in the plant tissues (particularly in the leaves) and 14.5-19.3% evolved as volatile components which were determined to be mainly carbon dioxide and acetonitrile.

Three main fractions were determined in the plant leaves (Table B.7.6): a polar fraction (0.3% applied radioactivity), a non-polar fraction (0.4%) and methomyl (2.8%). No significant radioactivity was found corresponding to metabolites methomyl oxime, methomyl sulphone (IN-M1284) or methomyl sulphoxide (IN-W1602). The polar and non-polar fractions were not investigated further.

Table B.7.7 shows the distribution of radioactivity in the plants treated by foliar application to a single leaf. Very little radioactivity was translocated to other parts of the plants 3 and 7 days after treatment. Most of the total radioactive residue was found in the treated leaf.

(Harvey, J.Jr., 1967, Harvey, J.Jr., Buchanan, J.B., 1968)

Table B.7.7 Distribution of [ $^{14}\text{C}$ ] - labelled methomyl equivalents in tobacco plant after foliar application to a single leaf.

Plant Tissues	3 days after application		7 days after application	
	$\mu\text{Ci}$	% of applied <sup>a</sup>	$\mu\text{Ci}$	% of applied <sup>a</sup>
Treated leaf	1.57	58.1	0.88 <sup>b</sup>	32.6
Growing tip	0.0	0	0.01	0.4
Leaves and stems above treated leaf	0.0	0	0.02	0.7
Leaves and stems below treated leaf	0.0	0	0.02	0.7
Roots	0.0	0	0.02	0.7

<sup>a</sup> Tobacco plants were treated with 2.70  $\mu\text{Ci}$  [ $^{14}\text{C}$ ]methomyl, % of applied calculated from this data

<sup>b</sup> Only 0.01  $\mu\text{Ci}$  (0.4%) was obtained from the surface water rinse of the leaf

*\*based on ref: Harvey J. et al., J. Agric. Food Chem., Vol. 5, pp. 769-774 (1973).*

### B.7.1.3 Cabbage

The metabolism of methomyl was investigated using [ $^{14}\text{C}$ ] -labelled methomyl (radiochemical purity >99%\*) in a study performed in 1968. A 42 day old cabbage plant (*cv* Burpee's Sure-head) grown in a 15 cm diameter pot was placed in a metabolism chamber. The upper surfaces of four leaves were treated with 325  $\mu\text{l}$  of a 2.6 mg/ml solution of [ $^{14}\text{C}$ ]methomyl dispensed by microsyringe. The chamber was connected to a series of three traps: first, a cold trap immersed in dry ice-trichloroethylene; second, a sodium hydroxide trap designed to trap acidic volatile compounds such as  $^{14}\text{C}$ -carbon dioxide. A third sodium hydroxide trap was placed after an oxidising furnace in order to trap substances that had been oxidised to  $\text{CO}_2$ . The contents of the cold trap were removed on the third and seventh day and analysed for radioactivity by LSC.

Seven days after the treatment the cabbage plant was cut off at ground level and macerated with four successive aliquots of methanol. The root was gently washed to remove any soil and allowed to air dry. The soil and the water used to wash soil from the roots were mixed thoroughly and allowed to air dry. Radioactivity in plant tissue extracts, volatile traps and condensate in the metabolism chamber were determined by LSC. The un-extracted radioactivity from plant tissue and radioactivity in the cabbage roots and soil were determined by LSC following combustion.

The methanol extracts from plant tissues were combined and concentrated to a single aqueous fraction. The non-aqueous deposit was dissolved in benzene. Separation of metabolite fractions was performed using a counter-current fractionator. Radioactivity in the fractions was analysed by LSC and characterisation performed by TLC.

The radioactivity in the first sodium hydroxide trap was examined by precipitation with barium chloride and analysed by LSC. The radioactivity in the dry ice-trichloro trap was partitioned between organic and aqueous phases and analysed by LSC and GC.

Table B.7.8 Distribution of [ $^{14}\text{C}$ ] - labelled methomyl equivalents in cabbage plant after foliar application

Component	$\mu\text{Ci}$	% dose <sup>a</sup>
<b>Plant Tissues</b>		
plant extract	2.293	51.8
plant residue	0.933	21.1
roots	0.035	0.8
<b>Volatile components</b>		
[ $^{14}\text{C}$ ]Carbon dioxide	0.343	7.8
[1- $^{14}\text{C}$ ]Acetonitrile <sup>b</sup>	0.334	7.6
<b>Condensate</b> <sup>c</sup>	0.195	4.4
<b>Growth media</b>		
soil	0.87	2.0
<b>Total Recovery</b>	4.220	95.5

<sup>a</sup> Cabbage plant were treated with 4.42  $\mu\text{Ci}$  [1- $^{14}\text{C}$ ]methomyl

<sup>b</sup> Volatiles from cold trap and post-furnace traps combined

<sup>c</sup> Condensed water in metabolism chamber

Table B.7.9 Composition of cabbage plant extracts after foliar application of [ $^{14}\text{C}$ ] - labelled methomyl

Countercurrent Fraction	Fraction number	$\mu\text{Ci}$ <sup>a</sup>	% Fraction <sup>b</sup>	% Applied radioactivity <sup>c</sup>
Polar fraction	0-6	1.03	62.4	23.3
	10-25	0.02	1.2	0.4
methomyl	30-60	0.11	6.7	2.5
Non-polar fraction	80-94	0.49	29.7	11.1
<b>Total Residues</b>		<b>1.65</b>	<b>100</b>	<b>37.3</b>

<sup>a</sup>  $\mu\text{Ci}$  recovered in each fraction

<sup>b</sup> % of extract found in these counter current fractions

<sup>c</sup> % of total radioactivity applied to cabbage plant (4.42 $\mu\text{Ci}$  total)

Table B.7.8 shows the distribution of radioactivity in the cabbage plant. Approximately 15% of the applied radioactivity was recovered as volatile components which were identified as carbon dioxide and acetonitrile. Radioactivity in the condensate fraction (4.4%) was identified as methomyl. Approximately 73% of the applied radioactivity was found in plant tissues with approximately 1% present in the roots. Extraction of the plant material yielded 51.8% of the applied radioactivity with 21.1% remaining as un-extracted residues.

Three main fractions were determined in the plant extracts (Table B.7.9): a polar fraction (23.7% applied radioactivity), a non-polar fraction (11.1%) and methomyl (2.5%). No significant radioactivity was found corresponding to metabolites methomyl oxime, methomyl sulphone (IN-M1284) or methomyl sulphoxide (IN-W1602).

Co-chromatography of the polar fraction by TLC systems with reference standards using various solvent systems revealed radioactivity associated with carboxylic acids, amino acids, glucose, sucrose and other sugars. Incubation of the polar fraction with  $\beta$ -glucosidase did not lead to the release of radioactivity associated with methomyl oxime. The non-polar fraction was characterised by saponification and partitioning into alkaline, aqueous (containing fatty acids) and ethyl ether soluble fractions. The alkaline fraction was derivatised and the resulting methyl esters were analysed by GC. The fraction was found to be composed of palmitic, palmitoleic, stearic and/or oleic and arachidic acids in a ratio of 5:2:3:3. The material in the ether phase was neutral and partially water-soluble. It did not partition into 10% potassium hydroxide or 1 N hydrochloric acid.

It was concluded that methomyl was rapidly degraded in cabbage to carbon dioxide and acetonitrile that volatilised from the plant tissues. Lipids, sugars and amino acids were identified or detected indicating that carbon fragments of methomyl were incorporated into natural products.

(Harvey, J., Yates, R.A., 1968a, Harvey, J., Reiser, R.W., 1968, Harvey, J., 1970)

*\*based on ref: Harvey J. et al., (1973), op. cit.*

### B.7.1.3 Maize

The metabolism of methomyl was investigated using [1- $^{14}$ C]-labelled methomyl (radiochemical purity >99 %\*) in a study performed in 1968. Four eleven day old maize plants (cv Eastern States 8-30) grown in a 6 inch diameter pot were placed in a metabolism chamber. 100  $\mu$ l of a 2.6 mg/ml solution of [1- $^{14}$ C]-methomyl were dispensed by pipette into the whorl at the growing point of each plant. The chamber was connected to a series of three traps: first, a cold trap immersed in dry ice-trichloroethylene; second, a sodium hydroxide trap designed to trap acidic volatile compounds such as  $^{14}$ C-carbon dioxide. A third sodium hydroxide trap was placed after an oxidising furnace in order to trap substances that had been oxidised to CO<sub>2</sub>. The contents of the cold trap were removed on the third and sixth day and analysed for radioactivity by LSC. The cold trap was removed after day six and the chamber was connected to the first of the sodium hydroxide traps.

Ten days after treatment the four plants were cut off at ground level, combined and macerated with four successive aliquots of methanol. The roots from the plants were gently washed to remove any soil, combined and then extracted with methanol as described above. The soil and the water used to wash soil from the roots were mixed thoroughly then centrifuged. The solid precipitate was allowed to air dry. Radioactivity in plant tissue extracts, volatile traps and condensate in the metabolism chamber were determined by LSC. The un-extracted radioactivity from plant tissue and radioactivity in the soil were determined by LSC following combustion.



The methanol extracts from plant tissues were combined and concentrated to a single aqueous fraction. The non-aqueous deposit was dissolved in benzene. Separation of metabolite fractions was performed using a counter-current fractionator. Radioactivity in the fractions was analysed by LSC and characterisation performed by TLC.

The radioactivity in the first sodium hydroxide trap was examined by precipitation with barium chloride and analysed by LSC. The radioactivity in the dry ice-trichloro trap was partitioned between organic and aqueous phases and analysed by LSC and GC.

Table B.7.10 Distribution of [ $^{14}\text{C}$ ] - labelled methomyl equivalents in maize plants 10 days after application

Component	$\mu\text{Ci}$	% dose <sup>a</sup>
<b>Plant Tissues</b>		
plant extract	1.537	28.3
plant residue	1.220	20.6
root extract	0.002	<0.1
root residue	0.019	0.3
<b>Volatile components</b>		
$^{14}\text{C}$ -Carbon dioxide <sup>b</sup>	0.373	6.9
[ $^{14}\text{C}$ ]Acetonitrile <sup>b</sup>	1.1474	26.5
<b>Condensate</b> <sup>c</sup>	0.732	13.5
Growth media		
soil Extract	0.34	0.6
soil residue	0.624	11.5
<b>Total Recovery</b>	5.885	108

<sup>a</sup> Maize plants were treated with 5.42  $\mu\text{Ci}$  [ $^{14}\text{C}$ ]methomyl

<sup>b</sup> Volatiles from cold trap and post furnace traps combined

<sup>c</sup> Condensed water in metabolism chamber

Table B.7.11 Composition of maize plant extracts after application of [ $^{14}\text{C}$ ] - labelled methomyl

Countercurrent fraction	Fraction number	$\mu\text{Ci}$ <sup>a</sup>	% Fraction <sup>b</sup>	% applied radioactivity <sup>c</sup>
Polar fraction	0-6	0.885	81.8	16.3
	10-25	0.015	1.4	0.3
methomyl	30-60	0.061	5.6	1.1
Non-polar fraction	80-94	0.121	11.2	2.2
<b>Total Residues:</b>		<b>1.082</b>	<b>100</b>	<b>19.9</b>

<sup>a</sup>  $\mu\text{Ci}$  recovered in each fraction

<sup>b</sup> % of extract found in these counter current fractions

<sup>c</sup> % of total-radioactivity applied to maize plants (5.44  $\mu\text{Ci}$ )

Table B.7.10 shows the distribution of radioactivity in the maize plants. Approximately 33% of the applied radioactivity was recovered as volatile components which were identified as carbon dioxide and acetonitrile. Radioactivity in the condensate fraction (13.5%) was identified as methomyl. Approximately 49% of the applied radioactivity was found in plant tissues with less than 1% present in the roots.

Extraction of the plant material yielded 28.3% of the applied radioactivity with 20.6% remaining as un-extracted residues.

Three main fractions were determined in the plant extracts (Table B.7.11): a polar fraction (16.3% applied radioactivity), a non-polar fraction (2.2%) and methomyl (1.1%). No significant radioactivity was found corresponding to metabolites methomyl oxime, methomyl sulphone (IN-M1284) or methomyl sulphoxide (IN-W1602).

Co-chromatography of the polar fraction by TLC systems with reference standards using various solvent systems revealed radioactivity associated with amino acids, glucose, sucrose and other sugars. Incubation of the polar fraction with  $\beta$ -glucosidase did not lead to the release of radioactivity associated with methomyl oxime. The non-polar fraction was characterised by saponification and partitioning into alkaline aqueous (0.4% of applied radioactivity, fatty acids) and ethyl ether (1.8% of applied radioactivity) soluble fractions. The material in the ether phase was neutral and partially water-soluble. It did not partition into 10% potassium hydroxide or 1 N hydrochloric acid. The majority of the radioactivity characterised was either found as methomyl or had been degraded to acetonitrile, carbon dioxide and / or other products of primary metabolism.

(Harvey, J., Yates, R.A., 1968b, Harvey, J., Reiser, R.W., 1968, Harvey, J., 1970)

*\*based on ref: Harvey J. et al., (1973) op. cit.*

### **B.7.1.3 Cotton**

A brief, two page paper was submitted. There was insufficient information contained in the paper to fully assess the data presented.

### **B.7.1.4 Metabolism, distribution and expression of the residue in succeeding crops**

No data were submitted. The notifier states that “Studies to determine residues of methomyl in succeeding crops are not required due to the short residual nature of methomyl in soil. Rate of degradation studies conducted in 3 soils at 20°C confirmed the DT<sub>50</sub> of methomyl to be 4-8 days. The major degradation route was mineralisation to <sup>14</sup>C-carbon dioxide. There were no significant metabolites (<3% of the applied radioactivity). The major hydrolysis product of methomyl, methomyl oxime, is short lived in soil, with a DT<sub>50</sub> of less than 1 day with the major metabolic product being <sup>14</sup>C-carbon dioxide. No significant residues of methomyl or metabolites, which could lead to residues above the limit of quantification at harvest, are expected to remain in soil or plant materials (e.g., straw or organic material) at the time of sowing or planting of succeeding crops.”

The longest DT<sub>90</sub> value for total extractable residues originating from labelled methomyl in soil is 43 days (section B.8.1.4) therefore significant residues in succeeding crops are unlikely.

**B.7.1.5 Summary/assessment**

The metabolism and distribution of methomyl was investigated in grapes using [ $^{14}\text{C}$ ]–labelled methomyl. A grapevine was treated with one application at a rate of 990 g ai/ha equivalent to a 2.2 N individual dose (1.1N maximum total dose). TRR were 1.282 mg/kg in grapes immediately after application falling to 0.942 mg/kg 14 days after treatment. For grapevine leaves TRR were initially 48.6 mg/kg and 21.2 mg/kg 14 days after application.

Methomyl was the major metabolite identified in grape berries accounting for 82.6% 51.4% TRR (0.485 mg/kg) in samples harvested 14 days after application. Other significant metabolites identified were methomyl oxime (7.2% TRR, 0.068 mg/kg) and IN-HUZ57 (5.6% TRR, 0.052 mg/kg). Approximately 11% TRR (0.102 mg/kg) were attributed to an unidentified polar fraction which was found to consist of at least 7 components none of which were found at levels greater than 3.1% TRR (0.03 mg/kg). No other metabolites were found in significant levels in the 14 day samples.

For grape foliage methomyl accounted for 3.6% TRR (0.763 mg/kg) 14 days after application. The major identified metabolite in the day 14 samples was IN-HUZ57 accounting for 16.4% TRR (3.468 mg/kg). No further characterisation work on the unextracted residue was performed.

Further studies on tobacco, cabbage and maize were submitted although these studies were not performed to modern standards and appear mainly to study the translocation and uptake of radio labelled methomyl as well as identify potential metabolites formed. No specific values for individual metabolites were provided for these studies – instead amounts were expressed as % applied radioactivity. In these studies the majority of applied radioactivity was broken down to carbon dioxide and acetonitrile or was bound to natural plant compounds (e.g. glucose), however significant amounts of applied radioactivity were not extracted from plant tissues. The studies found no evidence of the metabolite methomyl oxime in the radioactivity analysed. These studies were not considered as acceptable in order to propose a residue definition due to the concerns outlined above.

The grape metabolism study was considered as an acceptable study in order to propose a residue definition. Based on the metabolism data for grapes the most significant metabolite was methomyl; however significant levels of the metabolite methomyl oxime were found. It is considered that methomyl oxime is less toxic than the parent compound methomyl (see Section B.6.8.1) therefore a residue definition of methomyl only is proposed.

(Kennedy and Bentley, 2004).

No data were submitted to address residues in succeeding crops; this is considered acceptable.

**B.7.2 Metabolism, distribution and expression of the residues in livestock (AII 6.2, IIIA 8.1)****B.7.2.1 Cattle**

A study has not been submitted for cattle; a ruminant metabolism study is available for goats (B.7.2.2).

**B.7.2.2 Goats**

In a 1993 study one goat (Toggenburg/Alpine cross, 3-4 years old, body weight ~53 kg) was dosed orally with a mixture of [ $1\text{-}^{14}\text{C}$ ]-labelled and [ $1\text{-}^{13}\text{C}$ ]-labelled methomyl (radiochemical purity 98%) in capsules for three consecutive days. Each capsule contained 160 mg methomyl, equivalent to 162 mg/kg diet based on food consumption during the dosing period. The goat was housed in a metabolism cage fitted with a head restraint and chamber to enclose the head. The head chamber was connected to a series of traps designed to trap volatile compounds: three methanol traps (the first cooled by ice, followed with two cooled by dry ice) and a sodium hydroxide trap.

Urine and faeces were collected daily and milk twice daily. A decrease in body weight (ca10 percent) and milk production was noted during the dosing phase. This was thought to be due to the poor adaptation of the animal to confinement during the study. The goat was sacrificed approximately 22 hours after administration of the final dose and liver, kidneys, samples of fat (omental and renal) and samples of muscle (longissimus dorsi, triceps and gastrocnemius) were taken. Additional samples of blood, gall bladder contents, stomach (rumen, omasum and abomasum) and contents, small and large intestines and contents, cage rinse and drinking water were taken for material balance purposes. All samples were analysed by LSC or LSC following solubilisation. The distribution of radioactivity and levels of residues in milk and tissues are shown in Tables B.7.12 and B.7.13.

Table B.7.12 Distribution of radioactivity in milk and tissues of a goat dosed with [ $^{14}\text{C}$ ] - labelled methomyl

Specimen	Percent of total administered radioactivity
Total milk (0-72 hr)	3.22
Composite Muscle <sup>a</sup>	3.06
Composite Fat <sup>a</sup>	0.49
Liver	2.51
Kidney	0.18
Gall Bladder Contents	0.03
Gastrointestinal tract contents	7.5
Blood	2.65
Expired [ $^{14}\text{C}$ ]Acetonitrile	12.8
Expired $^{14}\text{CO}_2$	18.5
Urine	16.5
Faeces	5.0
Cage wash	0.4
Drinking water	6.3
Total	73.1

<sup>a</sup> Pooled tissue, total muscle mass and fat assumed to be 25% and 10% respectively of body weight

Table B.7.13 Residue levels in milk and tissues of a goat dosed with [ $^{14}\text{C}$ ] - labelled methomyl

Tissue	residues (mg/kg)
0-24 hr milk	4.09
24-48 hr milk	6.80
48-72 hr milk	9.31
Composite muscle <sup>a</sup>	1.45
Liver	12.1
Kidney	4.67
Fat <sup>b</sup>	0.32

<sup>a</sup> Composite muscle (longissimus dorsi, triceps and gastrocnemius)

<sup>b</sup> Composite fat (omental and renal)

Approximately 31% of the dose administered was expired as volatile metabolites, whilst ca 22% of the dose administered was excreted, mainly in urine. TRR in milk did not reach a plateau after 3 days. The highest levels of TRR in tissues were found in the liver

Tissue and milk samples were extracted with methanol: chloroform followed by two further aliquots of chloroform. The chloroform extracts were combined and the remainder extracted with methanol: water. After centrifugation the methanol: water fraction was removed and the residue was subjected to protein digestion using protease. Aliquots of the methanol: water extracts and protease supernatant were subjected to 6N acid hydrolysis under sealed vacuum. After cooling the vacuum was slowly vented into a sodium hydroxide trap. Aliquots of the chloroform extracts were subjected to saponification and partitioned into aqueous, alkaline (containing fatty acids) and ethyl ether soluble fractions. The alkaline fraction was derivatised and the resulting methyl esters were analysed by GC/MS. Sodium hydroxide traps were

precipitated with barium chloride and the radioactivity in the precipitate determined by LSC of the supernatant.

Characterisation and identification of metabolites was performed using HPLC - UV/ radiochemical detection against reference standards using various columns and mobile phases. Further identification was performed using LC-MS and NMR.

The extractability of the radioactive residues is shown in Table B.7.14 and the composition of the radioactivity is shown in Table B.7.15.

Table B.7.14 Extractability of radioactive residues in tissues of goats dosed with [<sup>14</sup>C] - labelled methomyl

Sample Type	Milk (48-72 hr)		Liver		Kidneys		Muscle		Fat	
	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR
TRR	9.31	100	12.1	100	4.67	100	1.45	100	0.32	100
Chloroform	4.92	52.8	1.65	13.6	1.50	32.1	0.68	46.9	0.14	43.8
Methanol/water	2.93	31.5	3.35	27.7	2.39	51.1	0.75	51.7	0.23	71.9
Protease solution	1.18	12.7	6.69	55.1	0.86	18.3	0.18	12.1	0.02	6.9
<b>Total extractable</b>	<b>9.03</b>	<b>97.0</b>	<b>11.69</b>	<b>96.4</b>	<b>4.75</b>	<b>101.7</b>	<b>1.61</b>	<b>111.0</b>	<b>0.39</b>	<b>121.9</b>
Post extraction solids	0.13	1.4	0.72	6.0	0.02	0.4	<0.01	<0.5	<0.01	<0.5

WARNING: This document forms part of an EC evaluation data package and should not be read in isolation. Reproduction must not be granted on the basis of this document.

Table B.7.15 Composition of radioactive residues in tissues of goats dosed with [<sup>14</sup>C] - labelled methomyl

Sample type	Milk (48-72 hr)		Liver		Kidneys		Muscle		Fat	
	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR
Acetonitrile (IN-07647)	1.730	18.6	1.240	10.2	1.423	30.5	0.902	62.2	0.127	39.7
Acetamide (IN-09066)	0.215	2.3	0.158	1.3	0.226	4.8	0.145	10.0	0.015	4.7
Saponifiable lipids <sup>a</sup>	2.900	31.1	0.240	2.0	0.058	1.2	0.018	1.2	0.022	6.9
Neutral lipids <sup>b</sup>	0.032	0.3	0.034	0.3	0.015	0.3	ND	ND	0.001	0.3
Water soluble phase after saponification <sup>c</sup>	0.240	2.6	0.340	2.8	0.048	1.0	0.016	1.1	0.009	2.8
Thiocyanate	1.610	17.3	0.850	7.0	1.620	34.7	0.250	17.2	0.160	50.0
Polar metabolites converted to acetic acid <sup>d</sup>	0.280	3.0	5.460	45.1	0.880	18.8	0.190	13.1	0.03	9.4
Polar metabolites determined as amino acids <sup>e</sup>	1.180	12.7	1.630	13.5	0.630	13.5	0.190	13.1	0.026	8.1
Lactose	0.870	9.3	NA	NA	NA	NA	NA	NA	NA	NA
<b>Total<sup>f</sup></b>	<b>8.841</b>	<b>95.0</b>	<b>9.79</b>	<b>80.9</b>	<b>4.674</b>	<b>100.0</b>	<b>1.566</b>	<b>107.9</b>	<b>0.375</b>	<b>117.2</b>
Unextracted	0.130	1.4	0.720	6.0	0.022	0.5	<0.01	<0.5	<0.01	<0.5

<sup>a</sup> Radioactivity recovered as capric, oleic/palmitic, stearic, lauric or myristic acids.

<sup>b</sup> Other radioactivity in neutral hexane extract.

<sup>c</sup> Radioactivity found in acidic water phase after extraction with dichloromethane.

<sup>d</sup> Summation of all polar metabolites found in methanol/water extracts and Protease digestion of tissue residues that was converted to acetic acid after HCl hydrolysis.

<sup>e</sup> Summation of all polar metabolites found in methanol/water extracts and Protease digestion of tissue residues that was found as glycine, serine, aspartic acid and glutamic acid after HCl hydrolysis.

<sup>f</sup> Total excludes acetamide, which would be included in acetic acid totals after acid hydrolysis.

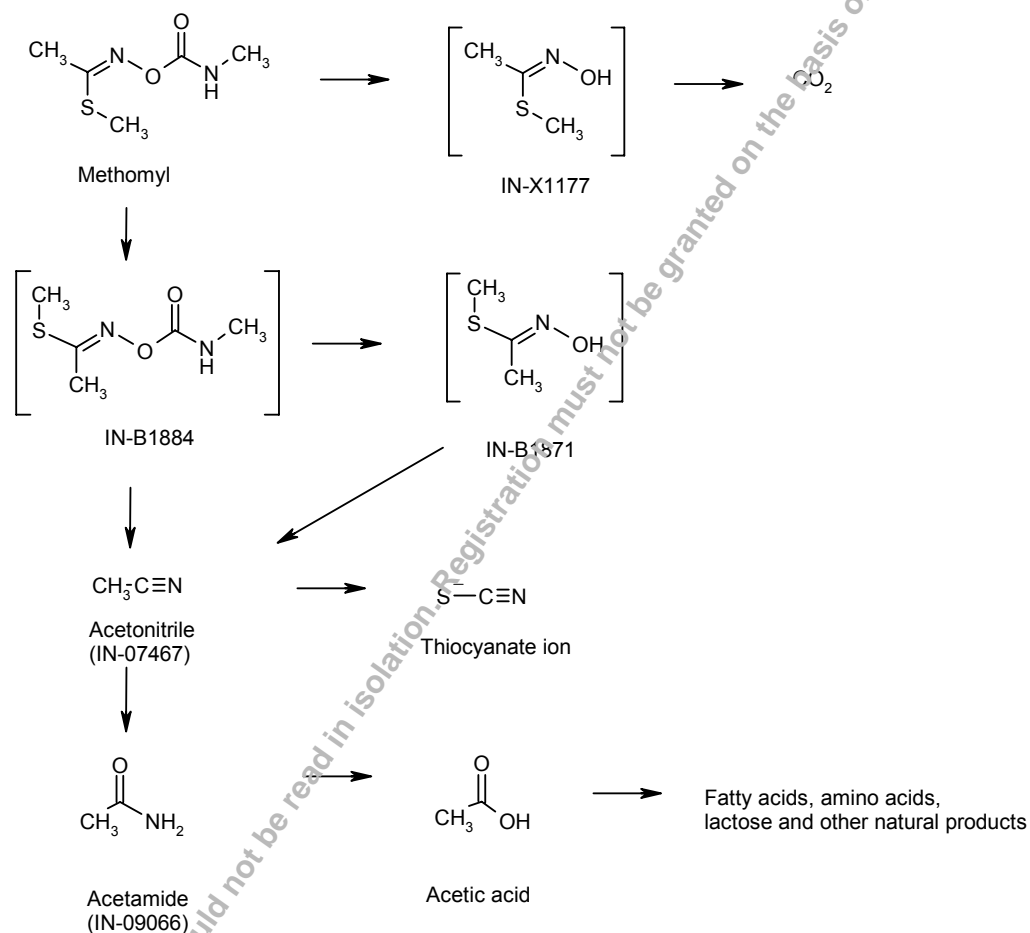
NA = not analysed, ND = not detected

Extractability of radioactivity was high in milk and tissues (> 80%). No residues of the parent methomyl were detected in any samples including the milk samples taken on days 1 and 2 of dosing. No metabolites closely related to the chemical structure of methomyl (methomyl sulphoxide, methomyl sulphone, hydroxymethyl methomyl and methomyl oxime) were detected, indicating that methomyl was extensively metabolised. Acetonitrile, acetamide and thiocyanate were detected in all samples.

Further characterisation of the radioactivity in tissues and milk indicated extensive metabolism of methomyl and acetonitrile and/or incorporation of radioactivity into natural products. A large part of total radioactivity in milk was incorporated in to natural products. Approximately 31% TRR were incorporated into fatty acids, 13% into amino acids, 9% into lactose and 0.3% into neutral lipids. This was also the case with edible tissues although to a lesser extent than in milk. The proposed metabolic pathway of methomyl in goat is shown in Figure B.7.2.

(Dietrich, R.F., Charlton, R.R., Ryan, D.L., McAleer, N.C. & Bookhart, S.W., 1995)

Figure B.7.2 Proposed metabolic pathway for methomyl in goat



[ ] postulated intermediates not detected

### B.7.2.3 Poultry

In a 1993 study, five hens (white leghorn) were dosed orally with a mixture of  $[1\text{-}^{14}\text{C}]$ -labelled and  $[1\text{-}^{13}\text{C}]$ -labelled methomyl (radiochemical purity >98%) in capsules for three consecutive days. Each capsule contained 5.1mg methomyl, equivalent to 45 mg/kg diet based on food consumption during the dosing period. The hens were housed in individual cages inside enclosed Plexiglas chambers. Air was pulled through the chambers and each chamber was connected to a series of traps designed to trap volatile compounds: three methanol traps (the first cooled by ice, followed with two cooled by dry ice) and a sodium hydroxide trap.

Excreta and cage rinses were collected daily. Eggs were collected twice daily, separated into whites and yolks and pooled. The contents of the traps were changed daily and retained for analysis. The hens were sacrificed approximately 22 hours after administration of the final dose and liver, fat and thigh and breast muscle samples were taken. The tissue samples from the five hens were pooled by type and were analysed by LSC or LSC following solubilisation. The distribution of radioactivity and levels of residues in eggs and tissues are shown in Tables B.7.16 and B.7.17.



Table B.7.16 Distribution of radioactivity in eggs and tissues of hens dosed with [ $^{14}\text{C}$ ] - labelled methomyl

Specimen	% of total administered radioactivity (SD) <sup>a</sup>
Muscle <sup>b</sup>	1.4
Fat <sup>b</sup>	0.8
Liver <sup>b</sup>	1.0
Total Eggs (0-72 hr)	1.1
Expired [ $^{14}\text{C}$ ]Acetonitrile	34.1 (± 3.7)
Expired $^{14}\text{CO}_2$	19.2 (± 3.3)
Excreta	25.8 (± 1.2)
Cage wash	1.5 (± 0.5)
Total	84.9

<sup>a</sup> Values as means of five replicate determinations on 5 hens for tissue residues, and mean ± standard deviation for excretion products

<sup>b</sup> Pooled tissue for all 5 hens, total muscle mass and fat assumed to be 25% and 10% respectively of body weight

Table B.7.17 Residue levels in eggs and tissues of hens dosed with [ $^{14}\text{C}$ ] - labelled methomyl

Tissue	residues (mg/kg)
0-24 hr Egg white	0.991
0-24 hr Egg yolk	0.462
24-48 hr Egg white	1.13
24-48 hr Egg yolk	0.900
48-72 hr Egg white	1.53
48-72 hr Egg yolk	1.94
Composite muscle <sup>a</sup>	0.54
Liver	2.97
Fat	0.794

<sup>a</sup> Breast and thigh muscle combined

Approximately 53% of the dose administered was expired as volatile metabolites, whilst ca 26% of the dose administered was excreted. TRR in eggs did not reach a plateau after 3 days. The highest levels of TRR in tissues were found in the eggs collected 48 – 72 hours after the first dose.

Liver, muscle, fat and egg samples were analysed for residue levels of methomyl, methomyl oxime, acetonitrile and acetamide within 48 hours of collection by extraction with methanol: water (1:1) and HPLC analysis.

Tissue and egg yolk samples were sequentially extracted with dichloromethane followed methanol: water (2:1, v/v). After centrifugation the methanol: water fraction was removed and the residue was subjected to protein digestion using protease. Aliquots of the methanol: water extracts and protease supernatant were subjected to 6N acid hydrolysis. Aliquots of the dichloromethane extracts were subjected to saponification and partitioned into aqueous, alkaline (containing fatty acids) and ethyl ether soluble fractions. The alkaline fraction was derivatised and the resulting methyl

esters were analysed by GC/MS. Sodium hydroxide traps were precipitated with barium chloride and the radioactivity in the precipitate determined by LSC of the supernatant.

Characterisation and identification of metabolites was performed using HPLC, UV/ radiochemical detection against reference standards using various columns and mobile phases. Further identification was performed using LC-MS and NMR.

The extractability of the radioactive residues is shown in Table B.7.18 and the composition of the radioactivity is shown in Table B.7.19.

Table B.7.18 Extractability of radioactive residues in tissues of hens dosed with [ $^{14}\text{C}$ ] - labelled methomyl

Sample Type	Egg White (48-72 hr)		Egg Yolk (48-72 hr)		Liver		Muscle		Fat	
	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR
TRR	1.53	100	1.94	100	2.97	100	0.540	100	0.794	100
Initial methanol/water	1.52	99.6	-	-	-	-	-	-	-	-
Dichloromethane	NP	NP	1.59	82.0	1.24	41.8	0.223	41.3	0.746	94.0
Methanol/water	NP	NP	0.071	3.7	0.326	11.0	0.066	12.2	0.014	1.8
Protease solution	NP	NP	0.154	7.9	1.08	36.4	0.216	40.0	0.030	3.8
<b>Total extractable</b>	<b>1.52</b>	<b>99.6</b>	<b>1.815</b>	<b>93.6</b>	<b>2.646</b>	<b>89.1</b>	<b>0.505</b>	<b>93.5</b>	<b>0.790</b>	<b>99.5</b>
Post extraction solids	0.05	3.3	0.128	6.6	0.318	10.7	0.036	6.7	0.004	0.5

NP = extraction not performed

WARNING: This document forms part of an EC evaluation data package and should not be read in isolation. Reproduction must not be granted on the basis of this document.

Table B.7.19 Composition of radioactive residues in tissues of hens dosed with [<sup>14</sup>C] - labelled methomyl

Sample type	Egg White (48-72 hr)		Egg Yolk (48 – 72 hr)		Liver		Muscle		Fat	
	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR
Acetonitrile (IN-07647)	1.37	89.5	0.417	21.5	0.289	9.7	0.140	25.9	0.056	7.1
Acetamide (IN-09066)	0.02	1.3	0.012	0.6	0.023	0.8	0.010	1.9	ND	ND
Oleic/palmitic acid	NA	NA	0.693	35.7	0.395	13.3	0.013	2.4	0.456	57.4
Stearic acid	NA	NA	0.111	5.7	0.056	1.9	0.003	0.6	0.042	5.3
Myristic acid	NA	NA	0.062	3.2	0.040	1.3	0.003	0.6	0.067	8.4
Other saponifiable lipids <sup>a</sup>	NA	NA	0.161	8.3	0.369	12.4	0.033	6.1	0.108	13.6
Cholesterol	NA	NA	0.087	4.5	0.095	3.2	NA	NA	NA	NA
Other non saponifiable lipids <sup>b</sup>	NA	NA	0.030	1.5	0.010	0.3	0.024	4.4	0.009	1.1
Water soluble phase after saponification <sup>c</sup>	NA	NA	0.030	1.5	ND	ND	0.007	1.3	0.007	0.9
Polar metabolites converted to acetic acid <sup>d</sup>	NA	NA	0.022	1.1	0.341	11.5	0.056	10.4	NA	NA
Polar metabolites determined as amino acids <sup>e</sup>	NA	NA	0.126	6.5	0.386	13.0	0.150	2.8	NA	NA
Other polar metabolites <sup>f</sup>	NA	NA	0.040	2.1	0.450	15.2	0.070	13.0	0.170	21.4
<b>Total</b>	<b>1.39</b>	<b>90.8</b>	<b>1.791</b>	<b>92.3</b>	<b>2.454</b>	<b>82.6</b>	<b>0.509</b>	<b>94.3</b>	<b>0.915</b>	<b>115.2</b>
Unextracted	0.05	3.3	0.123	6.6	0.318	10.7	0.036	6.7	0.004	0.5

<sup>a</sup> Other radioactivity saponified but not recovered as oleic, palmitic, stearic or myristic acids

<sup>b</sup> Other radioactivity in neutral hexane extract other than cholesterol

<sup>c</sup> Radioactivity found in acidic water phase after extraction with dichloromethane

<sup>d</sup> Summation of all polar metabolites found in methanol/water extracts and Protease digestion of tissue residues that was converted to acetic acid after HCl hydrolysis

<sup>e</sup> Summation of all polar metabolites found in methanol/water extracts and Protease digestion of tissue residues that was found as glycine, serine, aspartic and glutamic acids after HCl hydrolysis.

<sup>f</sup> Multiple unidentified metabolites none of which were greater than 4 %TRR or <0.1 mg/kg

NA = not analysed, ND = not detected

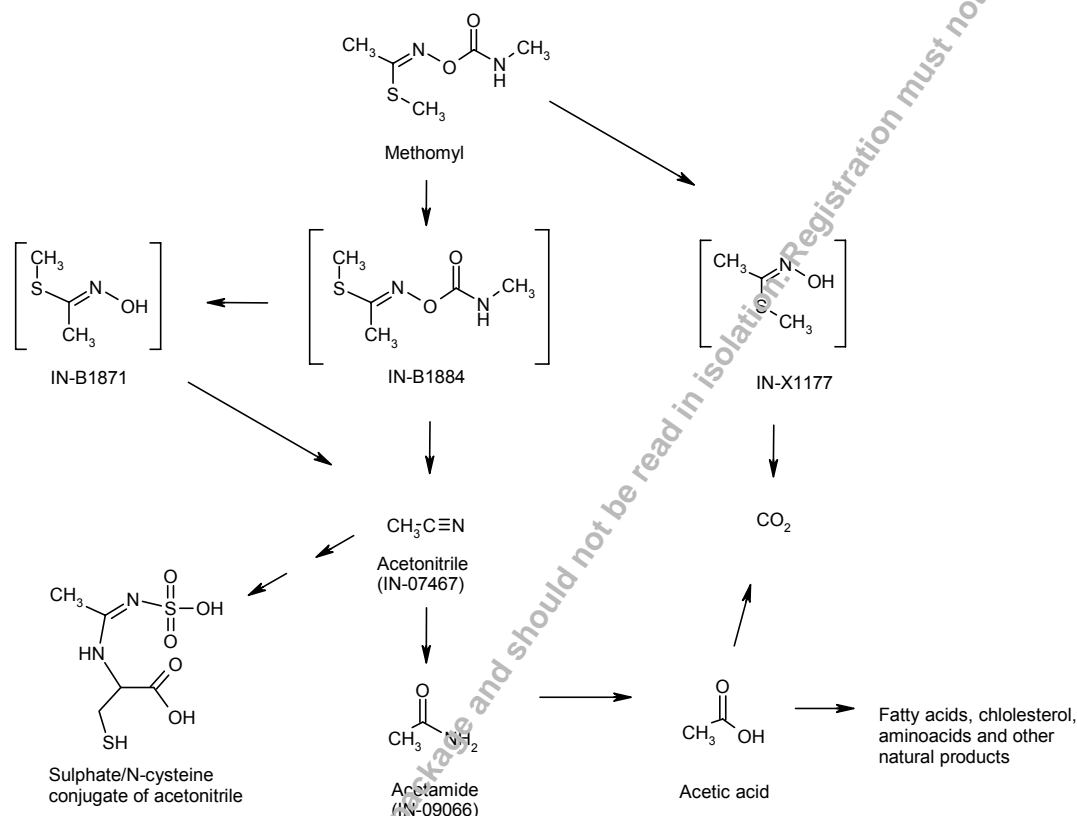
Extractability of radioactivity was high in all samples (> 89%). No residues of the parent methomyl were detected in any of the samples. No metabolites closely related to the chemical structure of methomyl (methomyl sulphoxide, methomyl sulphone, hydroxymethyl methomyl and methomyl oxime) were detected, indicating that methomyl was extensively metabolised.

Acetonitrile was the major single metabolite detected in all samples. In tissues and egg yolk significant amounts of radioactivity were incorporated into fatty acids (1.03mg/kg, 45.9% TRR for egg yolk, 0.858mg/kg, 28.9% TRR for liver and 0.05 mg/kg, 9.7% TRR for muscle), cholesterol (0.087 mg/kg, 4.5% TRR for egg yolk and 0.095 mg/kg, 3.2% TRR for liver) and amino acids (0.126 mg/kg, 6.5% TRR for egg yolk and 0.386 mg/kg, 13.0% TRR for liver).

Due to the extensive metabolism of methomyl, the unextractable residue (approximately 1-11% TRR) is considered likely to be the result of further incorporation of  $^{14}\text{C}$ -radiolabel into natural biological components. The proposed metabolic pathway of methomyl in laying hens is shown in Figure B.7.3.

(Djanegara, T.K.S. & Ryan, D.L., 1994)

Figure B.7.3 Proposed metabolic pathway for methomyl in laying hens



[ ] postulated intermediates not detected

#### B.7.2.4 Summary/assessment

The metabolism and distribution of methomyl was investigated in goats using  $[1-^{14}\text{C}]$  – labelled methomyl. One goat was treated with was dosed orally at a rate of 162 mg/kg diet for three consecutive days. The goat was housed in a metabolism cage fitted with a head restraint and chamber to enclose the head. The head chamber was connected to a series of traps designed to trap volatile compounds. Approximately 31% of the dose administered was expired as volatile metabolites, whilst ca 22% of the dose administered was excreted, mainly in urine. TRR in milk did not reach a plateau after 3 days. The highest levels of TRR in tissues were found in the liver.

Extractability of radioactivity was high in milk and all samples tissues ( $> 80\%$ ). No residues of the parent methomyl or metabolites closely related by structure to methomyl were detected in any samples including the milk samples taken on days 1

and 2 of dosing. Acetonitrile, acetamide and thiocyanate were detected in all samples. Further characterisation indicated extensive metabolism of methomyl and acetonitrile and/or incorporation of radioactivity into natural products.

Laying hens were dosed orally at a rate of 45 mg/kg diet for three consecutive days. The hens were housed in individual cages inside enclosed Plexiglas chambers. Air was pulled through the chambers and each chamber was connected to a series of traps designed to trap volatile compounds. Approximately 53% of the dose administered was expired as volatile metabolites, whilst ca 26% of the dose administered was excreted. TRR in eggs did not reach a plateau after 3 days. The highest levels of TRR in tissues were found in the eggs collected 48 – 72 hours after the first dose.

Extractability of radioactivity was high in all samples (> 89%). No residues of the parent methomyl or metabolites closely related by structure to methomyl were detected in any samples. Acetonitrile was the major single metabolite detected in all samples. Significant amounts of radioactivity were incorporated into fatty acids, cholesterol and amino acids indicating that methomyl was extensively metabolised. Due to the extensive metabolism of methomyl, the unextractable residue (approximately 1-11% TRR) is considered likely to be the result of further incorporation of the radiolabel into natural biological components.

Both the goat and poultry metabolism studies are considered acceptable under this evaluation as no uses on crops fed to animals have been identified. Further consideration of the data would be required if a future use on crops for animal feed is proposed.

### **B.7.3 Definition of the residue (IIA 6.2, IIIA 8.6)**

Based on the metabolism data submitted for grapevine, residues for monitoring purposes and risk assessment should be defined as methomyl.

Residues in succeeding crops are unlikely to be of significance. A residue definition for succeeding crops is not required.

A residue definition for animal products is not required.

WARNING: This document forms part of an EC evaluation data package and should not be used for regulatory purposes without the basis of this document.

**B.7.4 Use pattern**

Full details of registered Good Agricultural Practice are given in Volume 1, Section 1.5.4.

**B.7.5 Identification of critical GAPs**

Intended GAPs were identified as shown in Table B.7.20 (also Volume 1, Table 1.1).

On some occasions, two GAPs have been identified for the same crop since it is not obvious which uses/methods of application will give rise to the highest residue levels.

Table B.7.20 Summary of intended GAP

Crop and/or situation (a)	North (N) or South (S) Europe; Member State or Country	Product name	F G or I (b)	Pests or group of pests controlled (c)	Formulation		Application				Application rate per treatment			PHI (days) (l)
					Type (d-f)	Conc. of a.s. (i)	Method kind (g-h)	Growth stage & season (j)	Number min max (k)	Interval between applications (min days)	kg a.s./hL min max	Water L/ha min max	kg a.s./ha min max	
Cucumber/ Courgette	S Europe	Methomyl 20SL	F	Biting and sucking insects	SL	200g/L	MV/HV; foliar	Pre-harvest	1-2	14	0.025 - 0.09	500 - 1,000	0.25 - 0.45	7
Tomato/ Eggplant	S Europe	Methomyl 20SL	F	Biting and sucking insects	SL	200g/L	MV/HV; foliar	Pre-harvest	1-2	14	0.025 - 0.09	500 - 1,000	0.25 - 0.45	7
Grape (table & wine)	N. France; S Europe	Methomyl 20SL	F	Biting and sucking insects	SL	200g/L	HV; foliar	Pre-harvest	1-2	14	0.08 - 0.12	300 - 450	0.35	14
Grape (table & wine)	N. France; S Europe	Methomyl 20SL	F	Biting and sucking insects	SL	200g/L	HV; foliar	Pre-harvest	1-2	14	0.04 - 0.10	>450 - 1,200	0.45	14

## Remarks:

- (a) For crops, the EU and Codex classifications (both) should be used; where relevant, the use situation should be described (e.g., fumigation of a structure)
- (b) Outdoor or field use (F), glasshouse application (G), or indoor application (I)
- (c) e.g., biting/sucking insects, nematodes, soil born insects, foliar fungi, weeds
- (d) e.g., wettable powder (WP), emulsifiable concentrate (EC), granule (GR)
- (e) GCPF Codes – GIFAP Technical Monograph No. 2, 1989
- (f) All abbreviations must be explained
- (g) Method: high volume spraying, low volume spraying, spreading, dusting, drench;  
HV = high volume foliar spraying; MV = medium volume foliar spraying

- (h) Kind, e.g., overall, broadcast, aerial spraying, row, individual plant, between the plant – type of equipment used must be indicated.  
Foliar = foliar spraying
- (i) g/kg or g/L
- (j) Growth stage at last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application
- (k) Indicate the minimum and maximum number of application possible under practical conditions of use
- (l) PHI – minimum pre-harvest interval
- (m) Remarks may include: Extent of use/economic importance/restrictions

**B.7.6 Residues arising from supervised trials (IIA 6.3; IIIA 8.2)**

A summary of the trials is given in Table B.7.21. Results from trials conforming to GAP, reported in sufficient detail and acceptable analytical information are indicated by underlining. Where data form part of a decline trial, all data have been submitted but the sample which reflects critical GAP is marked by underlining.

Other trials may have been included where the reports lack sufficient detail. Although these reports cannot be directly used for recommending MRLs, they may be useful as supplementary information. Basic criteria for acceptability are given below:

Trials details

crop variety  
location, position and year of trial  
formulation used  
application rate  
maximum number of treatments  
growth stage of crop at treatment  
pre-harvest interval  
residue level (control and treated)

Analytical aspects

method specified and submitted  
storage of samples prior to analysis  
limit of determination  
acceptable recovery (70-110%)

-



Table B.7.21 A summary of supervised residue trials data generated

Crop/variety	Country/year	Formulation		Application					PHI (days)	Residue (mg/kg)	Comment	Ref.
		Type	Conc. of a.s	No.	Growth Stage	kg/ha (as)	Water (l/ha)	kg/hl (as)				
Grape vine/ Melon (white)	N. France/ 2000	SL	201.8 g/L	2	BBCH 81-83	0.442	389	0.114	14	<u>0.05</u>	13 days between applications	Nathan III, E.C., Dubey, L., Steiner, C., Mattou, H, 2001 Test site 7
					BBCH 83	0.471	518	0.091				
Grape vine/ Pinot noir (red)	N. France/ 2000	SL	201.8 g/L	2	BBCH 83	0.450	595	0.076	0	0.06	13 days between applications	Nathan III, E.C., Dubey, L., Steiner, C., Mattou, H, 2001 Test site 8
					BBCH 85	0.442	585	0.076	0+	0.34		
									1	0.25		
									3	0.22		
									7	0.05		
									14	0.03		
Grape vine/ Chardonnay (white)	N. France/ 2000	SL	201.8 g/L	2	BBCH 83	0.434	573	0.075	14	<u>0.21</u>	13 days between applications	Nathan III, E.C., Dubey, L., Steiner, C., Mattou, H, 2001 Test site 9
					BBCH 85	0.448	591	0.076				
Grape vine/ Chardonnay (white)	N. France/ 2000	WP	255.6 g/Kg	2	BBCH 83	0.464	605	0.077	14	<u>0.26</u>	13 days between applications	Nathan III, E.C., Dubey, L., Steiner, C., Mattou, H, 2001 Test site 9
					BBCH 85	0.460	600	0.077				

0 = before final application

0+ = after final application

Crop/variety	Country/year	Formulation		Application					PHI (days)	Residue (mg/kg)	Comment	Ref.
		Type	Conc. of a.s	No.	Growth Stage	kg/ha (as)	Water (l/ha)	kg/hl (as)				
Grape vine/ Cabernet franc (red)	N. France/ 2000	SL	201.8 g/L	2	BBCH 85	0.443	390	0.114	0	0.01	14 days between applications	Nathan III, E.C., Dubey, L., Steiner, C., Mattou, H, 2001 Test site 10
					BBCH 85	0.431	380	0.113	0+	0.24		
									1	0.18		
									3	0.10		
									7	0.05		
									14	0.02		
Grape vine/ Cabernet franc (red)	N. France/ 2000	WP	255.6 g/Kg	2	BBCH 85	0.434	378	0.115	0	0.01	14 days between applications	Nathan III, E.C., Dubey, L., Steiner, C., Mattou, H, 2001 Test site 10
					BBCH 85	0.460	400	0.115	0+	0.24		
									1	0.08		
									3	0.12		
									7	0.03		
									14	0.03		
Grape vine/ Cabernet Franc	N. France / 2001	SL	200.9 g/L	2	BBCH 83	0.445	394	0.113	0	0.07	13 days between applications	Nathan III, E.C., Dubey, L., Steiner, C., 2002 Test site 5
					BBCH 85	0.463	410	0.113	0+	0.70		
									1	0.61		
									3	0.47		
									7	0.38		
									14	0.20		

0 = before final application

0+ = after final application

Crop/variety	Country/year	Formulation		Application					PHI (days)	Residue (mg/kg)	Comment	Ref.
		Type	Conc. of a.s	No.	Growth Stage	kg/ha (as)	Water (l/ha)	kg/hl (as)				
Grape vine/ Chenin	N. France/ 2001	SL	200.9 g/L	2	BBCH 85	0.430	380	0.113	0	0.14	14 days between applications	Nathan III, E.C., Dubey, L., Steiner, C., 2002 Test site 6
					BBCH 85	0.451	399	0.113	0+	0.47		
									1	0.49		
									3	0.48		
									7	0.38		
									14	0.33		
Grape vine/ Pinot Noir	N. France/ 2001	SL	200.9 g/L	2	BBCH 83	0.441	294	0.150	14	0.06	15 days between applications	Nathan III, E.C., Dubey, L., Steiner, C., 2002 Test site 7
					BBCH 87	0.441	294	0.150				
Grape vine/ Pinot Meunier	N. France/ 2001	SL	200.9 g/L	2	BBCH 83	0.436	290	0.150	14	0.05	13 days between applications	Nathan III, E.C., Dubey, L., Steiner, C., 2002 Test site 8
					BBCH 87	0.446	296	0.151				
Grape vine / Roditis (white)	Greece/ 2000	SL	201.8 g/L	2	BBCH 86	0.457	1013	0.045	14	0.18	14 days between applications	Nathan III, E.C., Dubey, L., Steiner, C., Mattou, H, 2001 Test site 1
					BBCH 87	0.442	981	0.045				
Grape vine / Roditis (white)	Greece/ 2000	WP	255.6g/Kg	2	BBCH 86	0.459	1021	0.045	14	0.13	14 days between applications	Nathan III, E.C., Dubey, L., Steiner, C., Mattou, H, 2001 Test site 1
					BBCH 87	0.441	981	0.045				

0 = before final application

0+ = after final application

Crop/variety	Country/year	Formulation		Application					PHI (days)	Residue (mg/kg)	Comment	Ref.
		Type	Conc. of a.s	No.	Growth Stage	kg/ha (as)	Water (l/ha)	kg/hl (as)				
Grape vine/ Razaki	Greece/ 2000	SL	201.8g/L	2	BBCH 79	0.469	1040	0.045	0	0.12	14 days between applications	Nathan III, E.C., Dubey, L., Steiner, C., Mattou, H, 2001 Test site 2
					BBCH 85	0.427	952	0.045	0+	0.48		
									1	0.37		
									3	0.22		
									7	0.18		
								14	0.06			
Grape vine/ Cabernet Sauvignon (red)	Italy/ 2000	SL	201.8g/L	2	BBCH 83	0.454	1201	0.038	14	0.07	14 days between applications	Nathan III, E.C., Dubey, L., Steiner, C., Mattou, H, 2001 Test site 3
					BBCH 85	0.454	1201	0.038			Wine grapes	
Grape vine/ Cabernet Sauvignon (red)	Italy/ 2000	WP	255.6g/Kg	2	BBCH 83	0.457	1197	0.038	14	0.07	14 days between applications	Nathan III, E.C., Dubey, L., Steiner, C., Mattou, H, 2001 Test site 3
					BBCH 85	0.457	1197	0.038				
Grape vine/ Italia	Italy/ 2000	SL	201.8 g/L	2	BBCH 79	0.451	794	0.056	0		Trial discounted due to large variation in residues in duplicate samples	Nathan III, E.C., Dubey, L., Steiner, C., Mattou, H, 2001 Test site 4
					BBCH 85	0.475	838	0.057	0+	2.72, 0.03		
									1	1.03, 0.02		
									3	0.22, 0.89		
									7	0.58, 0.90		
									14	0.43, 0.63		

0 = before final application

0+ = after final application

Crop/variety	Country/year	Formulation		Application					PHI (days)	Residue (mg/kg)	Comment	Ref.
		Type	Conc. of a.s	No.	Growth Stage	kg/ha (as)	Water (l/ha)	kg/hl (as)				
Grape vine/ Semillon (white)	S. France/ 2000	SL	201.8 g/L	2	BBCH 85	0.449	296	0.152	14	<u>0.10</u>	14 days between applications	Nathan III, E.C., Dubey, L., Steiner, C., Mattou, H, 2001 Test site 5
					BBCH 85	0.443	293	0.151				
Grape vine/ Grenache (red)	S. France/ 2000	SL	201.8 g/L	2	BBCH 85	0.447	296	0.151	0	0.21	14 days between applications	Nathan III, E.C., Dubey, L., Steiner, C., Mattou, H, 2001 Test site 6
					BBCH 85	0.430	284	0.151	0+	0.68		
									1	0.50		
									3	0.50		
									7	0.36		
Grape vine/ Moschat d'Ambourg	Greece/ 2001	SL	200.9 g/L	2	BBCH 85	0.434	1158	0.037	14	<u>0.05</u>	14 days between applications	Nathan III, E.C., Dubey, L., Steiner, C., 2002 Test site 1
					BBCH 85	0.464	1238	0.037				
Grape vine/ Cabernet	Italy/ 2001	SL	200.9 g/L	2	BBCH 81	0.435	1156	0.038	0	0.14	15 days between applications	Nathan III, E.C., Dubey, L., Steiner, C., 2002 Test site 2
					BBCH 85	0.452	1200	0.037	0+	0.51		
									1	0.45		
									3	0.31		
									7	0.15		
									14	<u>0.11</u>		

0 = before final application

0+ = after final application

Crop/variety	Country/year	Formulation		Application					PHI (days)	Residue (mg/kg)	Comment	Ref.
		Type	Conc. of a.s	No.	Growth Stage	kg/ha (as)	Water (l/ha)	kg/hl (as)				
Grape vine/ Loin de l'Oeil	S. France / 2001	SL	200.9 g/L	2	BBCH 85	0.451	399	0.113	0	0.43	14 days between applications	Nathan III, E.C., Dubey, L., Steiner, C., 2002 Test site 3
					BBCH 85	0.474	419	0.113	0+	0.88		
									1	0.73		
									3	0.94		
									7	0.58		
Grape vine/ Sauvignon	S. France/ 2001	SL	200.9 g/L	2	BBCH 81	0.467	414	0.113	14	0.09	14 days between applications	Nathan III, E.C., Dubey, L., Steiner, C., 2002 Test site 4
					BBCH 85	0.470	416	0.113				

0 = before final application

0+ = after final application

Crop/variety	Country/year	Field or Glass house	Formulation		Application					PHI (days)	Residue (mg/kg)	Comment	Ref.
			Type	Conc. of a.s	No.	Growth Stage	kg/ha (as)	Water (l/ha)	kg/ hl (as)				
Cucumber/ Potomac F1	Italy/ 1996	F	SL	200 g/L	3	End flowering	0.490	980	0.05	0	<0.02	14 days between applications	Weidenauer, M., Françon, B., Larcinese, J.P, 1998. Test site 1
						Developm ent of fruits	0.485	970	0.05	0+	0.05		
						Mature fruits	0.520	1040	0.05	1	0.03		
										3	<0.02		
										5	<0.02		
Cucumber/ Potomac F1	Italy/ 1996	F	WP	250 g/Kg	3	End flowering	0.493	986	0.05	0	<0.02	14 days between applications	Weidenauer, M., Françon, B., Larcinese, J.P, 1998. Test site 1
						Developm ent of fruits	0.497	994	0.05	0+	0.05		
						Mature fruits	0.515	1030	0.05	1	0.05		
										3	<0.02		
										5	<0.02		
Cucumber/ Carine	Italy/ 1996	F	SL	200 g/L	3	Flowering	0.323	652	0.05	7	<0.02	5 and 14 days between applications	Weidenauer, M., Françon, B., Larcinese, J.P, 1998. Test site 2
						Young fruits	0.335	678	0.05				
						maturity	0.330	666	0.05				

0 = before final application

0+ = after final application

## Methomyl – Volume 3, Annex B: Residues

April 2004

Crop/variety	Country/year	Field or Glass house	Formulation		Application					PHI (days)	Residue (mg/kg)	Comment	Ref.
			Type	Conc. of a.s	No.	Growth Stage	kg/ha (as)	Water (l/ha)	kg/ hl (as)				
Courgette/	Italy/ 1996	F	SL	200 g/L	3	End flowering	0.496	992	0.05	7	<u>&lt;0.02</u>	21 and 14 days between applications No variety given	Weidenauer, M., Françon, B., Larcinese, J.P, 1998. Test site 3
						Growing fruits	0.515	1030	0.05				
						Mature fruits	0.520	1040	0.05				
Cucumber/ Ferri Morse	Greece/ 1996	F	SL	200 g/L	3	BBCH 69	0.500	997	0.05	7	<u>&lt;0.02</u>	21 and 14 days between applications	Weidenauer, M., Françon, B., Larcinese, J.P, 1998. Test site 4
						BBCH 73	0.500	1000	0.05				
						BBCH 83	0.500	994	0.05				
Cucumber/ Darina	Italy/ 1997	F	SL	200 g/L	3	BBCH 60	0.479	958	0.05	7	<u>&lt;0.02</u>	7 and 14 days between applications	Françon, B., Larcinese, J.P. , 1999  Test site 1
						BBCH 63	0.512	1023	0.05				
						BBCH 82	0.493	986	0.05				
Courgette/ Emeraude	S. France/ 1997	F	SL	200 g/L	3	BBCH 62	0.291	588	0.05	0	<0.02	15 days between applications	Françon, B., Larcinese, J.P. , 1999  Test site 2
						BBCH 63	0.305	609	0.05	0+	0.09		
						BBCH 65	0.306	612	0.05	1	0.03		
										3	<0.02		
										5	<0.02		
										7	<u>&lt;0.02</u>		

0 = before final application

0+ = after final application



Crop/variety	Country/year	Field or Glass house	Formulation		Application					PHI (days)	Residue (mg/kg)	Comment	Ref.
			Type	Conc. of a.s	No.	Growth Stage	kg/ha (as)	Water (l/ha)	kg/ hl (as)				
Courgette/ Emeraude	S. France/ 1997	F	WP	250 g/Kg	3	BBCH 62	0.304	608	0.05	0	<0.02	15 days between applications Trial discounted – residues < 0.02 mg/kg immediately after final application.	Fraçon, B., Larcinese, J.P. , 1999  Test site 2
						BBCH 63	0.301	602	0.05	0+	<0.02		
						BBCH 65	0.301	601	0.05	1	0.03		
										3 5 7	<0.02 <0.02 <0.02		
Courgette/ Verona	Greece/ 1997	F	WP	250 g/Kg	3	BBCH 82	0.251	501	0.05	0	<0.02	7 days between applications	Fraçon, B., Larcinese, J.P. , 1999  Test site 4
						BBCH 83-84	0.252	503	0.05	0+	0.03		
						BBCH 86-87	0.254	508	0.05	1	<0.02		
										3 5 7	<0.02 <0.02 <0.02		
Cucumber/ Potomac F1	Italy/ 1997	F	WP	250 g/Kg	3	BBCH 69	0.400	800	0.05	0	<0.02	8 and 13 days between applications	Fraçon, B., Larcinese, J.P. , 1999  Test site 5
						BBCH 71	0.399	798	0.05	0+	0.04		
						BBCH 85	0.405	810	0.05	1	<0.02		
										3 5 7	<0.02 <0.02 <0.02		

0 = before final application

0+ = after final application

Crop/variety	Country/year	Field or Glass house	Formulation		Application					PHI (days)	Residue (mg/kg)	Comment	Ref.
			Type	Conc. of a.s	No.	Growth Stage	kg/ha (as)	Water (l/ha)	kg/ hl (as)				
Tomato/ Red Setter	Italy/ 1996	F	SL	200 g/L	3	End flowering	0.485	970	0.05	0	<0.02	28 and 14 days between applications	Weidenauer, M., Françon, B., Larcinese, J.P., 1998 Test site 1
						Colour changing	0.501	1002	0.05	0+	0.04		
						Mature fruits	0.497	994	0.05	1	<0.02		
										3	<0.02		
										5	<0.02		
Tomato/ Red Setter	Italy/ 1996	F	WP	250 g/Kg	3	End flowering	0.497	994	0.05	0	<0.02	28 and 14 days between applications	Weidenauer, M., Françon, B., Larcinese, J.P., 1998 Test site 1
						Colour changing	0.505	1010	0.05	0+	0.07		
						Mature fruits	0.480	960	0.05	1	<0.02		
										3	<0.02		
										5	<0.02		
										7	<0.02		

0 = before final application

0+ = after final application

Crop/variety	Country/year	Field or Glass house	Formulation		Application					PHI (days)	Residue (mg/kg)	Comment	Ref.
			Type	Conc. of a.s	No.	Growth Stage	kg/ha (as)	Water (l/ha)	kg/ hl (as)				
Tomato/ Nemared	Spain/ 1996	F	SL	200 g/L	3	BBCH 65-71	0.304	605	0.05	0	<0.02	12 and 14 days between applications Trial discounted – residues < 0.02 mg/kg immediately after final application.	Weidenauer, M., Françon, B., Larcinese, J.P., 1998 Test site 3
						BBCH 66-80	0.276	551	0.05	0+	<0.02		
						BBCH 67-83	0.304	607	0.05	1	<0.02		
										3 5 7	<0.02 <0.02 <0.02		
Tomato/ Cannery row	Portugal/ 1996	F	SL	200 g/L	3	Mid flowering	0.363	725	0.05	7	<0.02	21 and 14 days between applications	Weidenauer, M., Françon, B., Larcinese, J.P., 1998 Test site 4
						Developm ent of fruits 5%	0.428	855	0.05				
						Mature fruits	0.483	965	0.05				
Tomato/ Erminia	Italy/ 1997	F	SL	200 g/L	3	BBCH 62	0.411	822	0.05	7	<0.02	27 and 14 days between applications	Françon, B., Larcinese, J.P., 1999 Test site 1
						BBCH 72	0.410	820	0.05				
						BBCH 83	0.389	778	0.05				
Tomato/ Erminia	Italy/ 1997	F	WP	250 g/Kg	3	BBCH 62	0.402	804	0.05	7	<0.02	27 and 14 days between applications	Françon, B., Larcinese, J.P., 1999 Test site 1
						BBCH 72	0.413	826	0.05				
						BBCH 83	0.418	836	0.05				

0 = before final application

0+ = after final application

## Methomyl – Volume 3, Annex B: Residues

April 2004

Crop/variety	Country/year	Field or Glass house	Formulation		Application					PHI (days)	Residue (mg/kg)	Comment	Ref.
			Type	Conc. of a.s	No.	Growth Stage	kg/ha (as)	Water (l/ha)	kg/hl (as)				
Tomato/ Empire	Spain/ 1997	F	WP	250 g/Kg	3	BBCH 62-71	0.434	858	0.05	7	<u>&lt;0.02</u>	12 and 14 days between applications	Fraçon, B., Larcinese, J.P., 1999 Test site 2
						BBCH 63-81	0.444	879	0.05				
						BBCH 65-82	0.449	888	0.05				
Tomato/ Heinz 8892	Portugal/ 1997	F	WP	250 g/Kg	3	BBCH 71	0.393	785	0.050	7	<u>&lt;0.02</u>	15 and 14 days between applications	Fraçon, B., Larcinese, J.P., 1999 Test site 3
						BBCH 78	0.445	891	0.050				
						BBCH 81	0.406	813	0.050				
Tomato/ Erminia	Italy/ 1997	F	SL	200 g/L	3	BBCH 62	0.401	802	0.05	0	<0.02	27 and 14 days between applications	Fraçon, B., Larcinese, J.P., 1999 Test site 4
						BBCH 72	0.409	818	0.05	0+	0.05		
						BBCH 83	0.406	812	0.05	1	0.02		
										3	<0.02		
										5	<0.02		
Tomato/ Empire	Spain/ 1997	F	WP	250 g/Kg	3	BBCH 62-72	0.465	920	0.05	0	<0.02	12 and 14 days between applications	Fraçon, B., Larcinese, J.P., 1999 Test site 5
						BBCH 68-75	0.442	873	0.05	0+	0.18		
						BBCH 69-83	0.448	885	0.05	1	0.03		
										3	<0.02		
										5	<0.02		
										7	<0.02		

0 = before final application

0+ = after final application

**B.7.6.3 Summary of residues resulting from supervised trials**

A summary of the residues trials provided for methomyl is given in table B.7.21. Trials were performed on the following crops: grapevine, cucurbits (cucumber and courgette), and Solanaceae (tomato). All trials were performed in the field. Trials were carried out using either a 200 g/l soluble concentrate formulation or a 250 g/kg wettable powder formulation. The difference in these formulation types is not considered to have a significant impact on the residues found.

For grapevines a total of 22 trials were conducted over 2 seasons: 10 in the north and 12 in the south. For each trial 2 foliar applications were made at a target rate of 0.45 kg ai/ha. Residues of methomyl were found in all trials in the range 0.02 – 0.59 mg/kg. There was no significant difference between residues found in trials from the north and those from the south. For four trials applications were made at rates outside the proposed GAP, however the residues found in these trials were comparable with those from trials performed according to the proposed GAP and so the data were considered acceptable. One trial performed in Italy in 2000 was discounted due to a poor agreement between duplicate results. Samples were stored at <18°C for up to 9 months prior to analysis.

For cucurbits a total of 10 trials were conducted over 2 seasons in the south. All trials were conducted in the field. 6 of the trials were performed on cucumber and 4 of the trials on courgette. For each trial 3 foliar applications were made at a target rate of 0.50 kg ai/ha. The proposed GAP is for a maximum of two applications therefore all the trials have been overdosed. Given that the decline data show that residues are relatively non-persistent in these crops it is considered that the trials are acceptable. In all the trials no residues of methomyl were found above the LOQ value of 0.02 mg/kg in samples with a 7 day PHI. For one decline trial on courgettes residues of methomyl were < 0.02 mg/kg immediately after the final application therefore this trial was discounted. Samples were stored at <18°C for up to 16 months prior to analysis.

For Solanaceae a total of 10 trials were conducted over 2 seasons in the south. All trials were conducted in the field and were performed on tomato. For each trial 3 foliar applications were made at a target rate of 0.25 – 0.50 kg ai/ha. The proposed GAP is for a maximum of two applications therefore all the trials have been overdosed. Given that the decline data show that residues are relatively non-persistent in these crops it is considered that the trials are acceptable. In all the trials no residues of methomyl were found above the LOQ value of 0.02 mg/kg in samples with a 7 day PHI. For one decline trial residues of methomyl were < 0.02 mg/kg immediately after the final application therefore this trial was discounted. Samples were stored at <18°C for up to 17 months prior to analysis. Sufficient trials data were submitted to support use on tomato and extrapolation to aubergine.

Table B.7.22 Summary of residue trials data for methomyl in various crops

Crop	GAP	No of trials relevant to GAP	Range of residues (mg/kg)	STMR (mg/kg)
Grapevine (Northern Europe)	2 x 0.35 kg ai/ha, OR 2 x 0.45 kg ai/ha, PHI = 14	10	0.02 – 0.33	0.055
Grapevine (Southern Europe)	2 x 0.35 kg ai/ha, OR 2 x 0.45 kg ai/ha, PHI = 14	11	0.06 – 0.59	0.10
Grapevine (Northern & Southern Europe combined)	As above	21	0.02 – 0.59	0.090
Cucumber/Courgette (Southern Europe)	2 x 0.45 kg ai/ha, PHI = 7	9	<0.02 - <0.02	0.02
Tomato/Aubergine (Southern Europe)	2 x 0.45 kg ai/ha, PHI = 7	9	<0.02 - <0.02	0.02

### B.7.7 Stability of residues prior to analysis

#### B.7.7.1 Grapes

Homogenised samples of seedless grapes were fortified with methomyl at 5.0 mg/kg and then stored at -20°C. Analysis for methomyl was performed at day 0 prior to freezing and at intervals up to 27 months. Analytical recoveries for samples fortified at 5.0 mg/kg were in the range 70 - 110% and were acceptable. The freezer storage stability data for grapes are shown in Table B.7.23. The data show no significant degradation of residues of methomyl in grapes over 27 months of storage at -20°C.

(Kennedy, C.M. & Tomic, D.M., 1993)

Table B.7.23 A summary of freezer storage stability data in grapes.

Matrix	Storage time (months)	Residue (mg/kg)	Storage recoveries (%)	Procedural recoveries (%)
Grapes	0	4.13	83	77
	1	4.03	81	78
	3	3.90	78	78
	6	3.88	78	78
	9	4.03	81	87
	12	4.10	82	81
	18	4.04	81	84
	24	3.45	69	78
	27	4.23	85	90

**B.7.7.2 Processed grape fractions**

Homogenised samples of grapes, raisins, grape juice and wine were fortified with methomyl at 0.1 mg/kg and then stored at  $\leq -18^{\circ}\text{C}$ . Analysis for methomyl was performed at day 0 prior to freezing and at intervals up to 11 months. Analytical recoveries for samples fortified at 0.1 mg/kg were in the range 70 - 110% except for one recovery for the grape juice samples, and were acceptable. The freezer storage stability data are shown in Table B.7.24. The data show no significant degradation of residues of methomyl in grapes, raisins and grape juice over 8 months of storage at  $-18^{\circ}\text{C}$  and wine over 11 months of storage at  $-18^{\circ}\text{C}$ .

(Nathan III, E.C., Dubey, L., Steiner, C. & Mattou, H., 2002)

Table B.7.24 A summary of freezer storage stability data in grapes and processed grape fractions.

Matrix	Storage time (months)	Residue (mg/kg)	Storage recoveries (%)	Procedural recoveries (%)
Grapes	0	0.089	89	89
	1	0.087	87	86
	2	0.085	85	84
	3	0.085	85	87
	5	0.089	89	88
	8	0.088	88	79
Raisin	0	0.092	92	92
	1	0.096	96	95
	2	0.098	98	97
	3	0.089	89	96
	5	0.097	97	97
	8	0.091	91	101
Grape Juice	0	0.087	87	87
	1	0.091	91	115
	2	0.080	80	107
	3	0.077	77	92
	5	0.087	87	102
	8	0.073	73	90
Wine	0	0.093	98	98
	1	0.099	99	109
	2	0.080	80	96
	3	0.085	85	94
	5	0.086	86	88
	8	0.083	83	94
	11	0.078	78	89

**B.7.7.3 Broccoli**

Homogenised samples of broccoli were fortified with methomyl at 2.0 mg/kg and then stored at -20°C. Analysis for methomyl was performed at day 0 prior to freezing and at intervals up to 24 months. Analytical recoveries for samples fortified at 2.0 mg/kg were in the range 70 - 110% and were acceptable. The freezer storage stability data for broccoli are shown in Table B.7.25. The data show no significant degradation of residues of methomyl in broccoli over 24 months of storage at -20°C.

(Kennedy, C.M. & Devine, P.G., 1993)



Table B.7.25 A summary of freezer storage stability data in broccoli.

Matrix	Storage time (months)	Residue (mg/kg)	Storage recoveries (%)	Procedural recoveries (%)
Broccoli	0	1.81	91	95
	1	1.71	86	85
	3	1.69	85	80
	6	1.81	91	95
	9	1.86	93	100
	12	1.97	99	100
	18	1.60	80	90
	24	1.84	92	110

**B.7.7.4 Lettuce**

Homogenised samples of lettuce were fortified with methomyl at 5.0 mg/kg and then stored at -20°C. Analysis for methomyl was performed at day 0 prior to freezing and at intervals up to 24 months. Analytical recoveries for samples fortified at 5.0 mg/kg were in the range 70 - 110% and were acceptable. The freezer storage stability data for lettuce are shown in Table B.7.26. The data show no significant degradation of residues of methomyl in lettuce over 24 months of storage at -20°C.

(Kennedy, C.M. & Devine, P.G., 1993)

Table B.7.26 A summary of freezer storage stability data in lettuce.

Matrix	Storage time (months)	Residue (mg/kg)	Storage recoveries (%)	Procedural recoveries (%)
Lettuce	0	4.80	96	88
	1	4.48	90	88
	3	4.90	98	90
	6	3.49	70	70
	6.5	3.68	74	74
	9	4.75	95	94
	12	4.48	90	84
	18	4.95	99	108
	24	4.48	90	84

**B.7.7.5 Potato, Bean Seed and Peanut Nutmeats.**

Potato tubers, peanut nutmeats and shelled pinto beans were placed on raised screened panels in a single layer and treated with a single broadcast spray of a water soluble powder formulation of methomyl. Potato tubers were treated at a rate of 0.25kg ai/ha and beans and peanuts at a rate of 0.04 kg ai/ha. After 24 hours samples of potatoes, beans and peanuts were frozen at -20°C. Each sample of potatoes consisted of 24 tubers and 1 kg of beans and peanuts were taken for each sample. Samples were homogenised and analysed at least in triplicate for methomyl at day 0 and at intervals up to 26 months. Analytical recoveries for samples fortified at 0.08 and 0.50 mg/kg were in the range 70 - 110% and were acceptable with the exception of the recoveries for the 26 month bean samples. The freezer storage stability data are shown in Table B.7.27. The data show no significant degradation of residues of methomyl in potato, bean seed and peanut over 26 months of storage at -20°C.

In addition homogenised samples were fortified with methomyl at 0.1 or 0.2 mg/kg and frozen at -20°C. Samples were taken for analysis at intervals up to 26 months, however no initial day 0 values were obtained. The data for this experiment are not presented.

(Milby, K.H., 2000)

WARNING: This document forms part of an EC evaluation data package and should not be read in isolation. Registrants must not base their application on the basis of this document.

Table B.7.27 A summary of freezer storage stability data in potato, bean seed and peanut.

Matrix	Storage time (months)	Mean Residue (mg/kg)	% of 0-month result	Procedural recoveries (%)
Potato	0	0.47 (n=6)	-	84, 91
	1	0.45 (n=3)	96	90, 91
	3	0.41 (n=3)	87	80, 88
	6	0.41 (n=3)	87	84, 77
	12	0.41 (n=3)	87	94, 87
	18	0.56 (n=3)	119	78, 92
	26	0.44 (n=3)	94	78, 90
Bean Seed	0	0.34 (n=6)	-	85, 90
	1	0.46 (n=3)	135	91, 90
	3	0.33 (n=3)	97	71, 83
	6	0.35 (n=3)	103	80, 88
	12	0.28 (n=3)	82	83, 83
	18	0.33 (n=3)	97	78, 95
	26	0.31 (n=3)	91	39, 68
Peanut	0	0.44 (n=6)	-	90, 97
	1	0.51 (n=3)	116	106, 84
	3	0.46 (n=3)	105	98, 96
	8	0.30 (n=3)	68	108, 96
	12	0.44 (n=3)	100	89, 105
	18	0.53 (n=3)	120	71, 97
	26	0.30 (n=3)	68	84, 96

**B.7.7.6 Maize**

Homogenised samples of maize kernel were fortified with methomyl at 0.1 mg/kg and then stored at -20°C. Analysis for methomyl was performed at day 0 prior to freezing and at intervals up to 24 months. Analytical recoveries for samples fortified at 0.1 mg/kg were in the range 65 - 110%. The freezer storage stability data for maize are shown in Table B.7.28. The data for this study are variable with only 74% recovered on day 0. No conclusions about the stability of methomyl in maize can be drawn from this study.

(Rühl, J.C., Devine, P.G., 1994)

Table B.7.28 A summary of freezer storage stability data in maize.

Matrix	Storage time (months)	Residue (mg/kg)	Storage recoveries (%)	Procedural recoveries (%)
Maize	0	0.074	74	80
	1	0.070	70	92
	3	0.062	62	84
	6	0.074	74	65
	9	0.054	54	89
	12	0.072	72	81
	18	0.069	69	65
	19.5	0.077	77	74
	24	0.072	72	82

**B.7.7.6 Animal products**

Homogenised samples of cows' milk, liver and muscle were fortified with methomyl at 0.1 mg/kg and then stored at  $-70^{\circ}\text{C} \pm 10^{\circ}\text{C}$ . Analysis for methomyl was performed using a matrix solid phase dispersion method with RP-HPLC- fluorescence detection at day 0 and at intervals up to 181 days for milk and muscle, and intervals up to 165 days for liver. Analytical recoveries for samples fortified at 0.1 mg/kg were in the range 70 - 110% and were acceptable. The freezer storage stability data are shown in Table B.7.29. The data show no significant degradation of methomyl residues in milk and muscle stored for 181 days and for liver stored for 165 days at  $-70^{\circ}\text{C} \pm 10^{\circ}\text{C}$ .

(Daun, R.J., 1995)

Table B.7.29 A summary of freezer storage stability data in cows' milk, liver and muscle.

Matrix	Storage time (days)	Residue (mg/kg)	Storage recoveries (%)	Procedural recoveries (%)
Milk	0	0.079	79	79
	30	0.099	99	91
	61	0.083	83	90
	91	0.085	85	84
	181	0.087	87	88
Liver	0	0.095	95	95
	11	0.102	102	108
	20	0.093	93	95
	31	0.088	88	90
	60	0.094	94	92
	90	0.094	94	99
	165	0.094	94	87
Muscle	0	0.085	85	85
	9	0.093	93	93
	15	0.089	89	83
	33	0.104	104	100
	61	0.090	90	88
	91	0.091	91	100
	181	0.088	88	97

#### B.7.7.8 Summary/assessment

Acceptable storage stability data were submitted for grape, processed grape fractions; broccoli, lettuce, potato, bean seed, peanut, milk, liver and muscle (see Table B.7.30) for methomyl. The storage stability data provided support the analysis of methomyl for residues trials and processing data submitted for grapes and processed grape fractions. No specific freezer storage data were provided for tomato, cucumber and courgette however sufficient storage data on other crops were provided to support the residue trials data. The storage stability data for animal products support the data generated for methomyl in livestock feeding studies.

Table B.7.30 Summary of acceptable freezer storage stability data for methomyl.

Matrix	Temperature	Storage period
Grape	-20°C.	27 months
Grape	-18°C.	8 months
Raisin	-18°C.	8 months
Grape juice	-18°C.	8 months
Wine	-18°C.	11 months
Broccoli	-20°C.	24 months
Lettuce	-20°C.	24 months
Potato	-20°C.	26 months
Bean seed	-20°C.	26 months
Peanut	-20°C.	26 months
Milk	-70°C.	181 days
Liver	-70°C.	165 days
Cows' muscle	-70°C.	181 days

### B.7.8 Effects of industrial processing and/or household processing (IIA 6.5, IIIA 8.4)

#### B.7.8.1 Effects on the nature of the residue

A study was carried out to simulate the conditions of pasteurisation, sterilisation and baking, brewing and boiling. Studies were performed using [ $^{14}\text{C}$ ] - 1 labelled methomyl (radiochemical purity > 95%) which was dissolved in methanol and added to aqueous buffer solutions at pH 4, 5 and 6 at a concentration of *ca* 8 µg/ml. The concentration of organic solvent in the resulting buffer solutions was 0.2%. The glassware containing the fortified buffer solutions were sealed and wrapped in aluminium foil to avoid the influence of light. The solutions were incubated at 90°C, 100°C or 120°C for up to 60 minutes.

Aliquots of the solutions were analysed by LSC and HPLC with UV and radiochemical detection at time zero and after incubation. Reference standards were used to identify any potential metabolites formed. A summary of the conditions and results is given in Table B.7.31. (This study is also summarised in Section B.8.4.1b)

Table B.7.31 Summary of the hydrolysis study to investigate the effect on methomyl residues

Process	Test Conditions	% Applied Radioactivity			
		Before Test		After Test	
		methomyl	methomyl oxime	methomyl	methomyl oxime
Pasteurisation	pH 4, 90 °C, 20 min	100	0	93.7	4.3
Baking, brewing and boiling	pH 5, 100 °C, 60 min	100	0	86.5	13.5
Sterilisation	pH 6, 120 °C, 20 min	100	0	58.2	39.5

Following simulated pasteurisation 93.7% of the applied radioactivity consisted of methomyl. An additional 4.3% of the applied radioactivity was determined to be the metabolite methomyl oxime. After the simulated baking, brewing, and boiling methomyl declined to 86.5% of the applied radioactivity. The major degradation product was determined to be methomyl oxime, at 13.5%. Following simulated sterilisation methomyl declined to 58.2% of the applied radioactivity, with 39.5% of the applied radioactivity determined as methomyl oxime.

Methomyl remains the primary analyte after exposure to simulated pasteurisation, baking, brewing, and boiling and sterilisation conditions of processing. The proportion of the metabolite methomyl oxime formed increased with increasing pH and temperature. It is considered that in some cases there may be significant residues of methomyl oxime in processed products.

(Pedersend, C.T., 2001)

#### B.7.8.2 Effects on residue levels

Processing studies were performed on grape samples taken from four of the residue trials to produce red grape juice and wine, white grape juice and wine and raisins. Samples of the processed products and intermediates from the processing procedures were analysed for methomyl. A summary of the residues and processing factors is given in Table B.7.32.

Table B.7.32 Summary of the methomyl residues found in grape processing fractions.

Raw commodity	Residue (mg/kg)	Processing method	Processed fractions	Residue (mg/kg)	Transfer factor
Whole grapes	0.10	White wine production	Must	0.079	0.76
			Wet pomace	0.120	1.10
			Dry pomace	0.023	0.22
			Must deposit	0.100	0.96
			Young wine	0.100	0.95
			Wine	0.084	0.80
		Juice production	Wet pomace	0.053	0.51
			Juice	0.012	0.11
		Raisin production - drying	Raisin	<0.01	0.10
Whole grapes	0.097	Red wine production	Must	0.130	1.29
			Wet pomace	0.040	0.40
			Dry pomace	0.071	0.73
			Young wine	0.033	0.34
			Lees	<0.01	0.10
			Wine	<0.01	0.10
		Juice production	Wet pomace	0.16	1.65
			Juice	0.015	0.15
		Raisin production - drying	Raisin	<0.01	0.10
Whole grapes	0.038	White wine production	Must	0.046	1.20
			Wet pomace	0.073	1.95
			Dry pomace	0.028	0.73
			Must deposit	0.060	1.59
			Young wine	0.046	1.23
			Wine	0.042	1.11
		Juice production	Wet pomace	0.045	1.20
			Juice	0.010	0.27
		Raisin production - drying	Raisin	<0.01	0.27
Whole grapes	0.031	Red wine production	Must	0.028	0.89
			Wet pomace	0.014	0.45
			Dry pomace	<0.01	0.32
			Young wine	0.011	0.32
			Lees	<0.01	0.32
			Wine	<0.01	0.32
		Juice production	Wet pomace	0.022	0.71
			Juice	<0.01	0.32
		Raisin production - drying	Raisin	<0.01	0.32



A mass balance study was performed using the data from one of the white wine production processed as shown in table B.7.33.

Table B.7.33 Mass balance of methomyl residues in processed white wine grape fractions.

Grapes	Matrix	Total weight of matrix (kg) <sup>a</sup>	methomyl concentration per matrix (mg/kg)	Total methomyl residue in matrix (mg) <sup>b</sup>	% methomyl residue distribution per matrix	% Mass balance <sup>c</sup>
						100*(C+D+F)/A
white	A grapes	59.85	0.038	2.27	100	127
	B must	34.30	0.046	1.58	70	
	C wet pomace	25.30	0.073	1.85	81	
	D must deposit	4.20	0.060	0.252	11	
	E AF wine	26.45	0.046	1.22	54	
	F wine	18.65	0.042	0.783	34	

<sup>a</sup> Weight for grapes is the starting weight of grapes processed.

<sup>b</sup> Total methomyl residue in matrix (mg) = [total weight of matrix through processing (kg)] x [methomyl concentration in matrix (mg/kg)]

<sup>c</sup> Calculations are performed using non-rounded values to determine the percent recovery to the nearest whole number.

(Nathan III, E.C., Dubey, L., Steiner, C. & Mattou, H., 2001)

No processing studies were performed on tomato, cucumber and courgette samples as no significant residues were found in the residues trials.

### B.7.8.3 Summary/assessment

Studies undertaken in processed grape fractions indicated that the majority of processing factors for commodities for human consumption were less than 1.0.

A hydrolysis study showed that methomyl remains the primary analyte after exposure to simulated pasteurisation, baking, brewing, and boiling and sterilisation conditions of processing, however significant proportions of the metabolite methomyl oxime were formed with increasing pH and temperature. It is considered that in some cases there may be significant residues of methomyl oxime in processed products, however methomyl oxime is considered less toxic than the parent compound (see Section B.6.8.1).

No processing studies were performed on tomato, cucumber and courgette samples as no significant residues were found in the residues trials.

### B.7.9 Livestock feeding studies (IIA 6.4, IIIA 8.3)

Holstein cows were dosed with methomyl daily at a rate of 8, 34 and 86 mg/kg feed (dry matter), equivalent to 0.29, 1.24 and 3.13 mg/kg bw/day for 28 consecutive days by capsule using a balling gun. Three animals were used for each dose rate. Milk samples

were taken twice daily. Aliquots of the milk samples taken on day 14 were centrifuged to give cream and skimmed milk samples. The animals were sacrificed within 21 hours of the final dose and samples of muscle (triceps, gracilis and longissimus dorsi), liver, kidney and fat (omental, renal and subcutaneous) were taken for analysis. Dosing did not have a significant effect on animal body weights or milk production.

Samples of milk and tissues were analysed for residues of methomyl by a matrix solid phase dispersion method with RP-HPLC- fluorescence detection. All samples were analysed within 181 days of freezer storage. Storage stability data for freezer storage of milk and tissues were provided and are discussed in Section B.7.7.7. Procedural recoveries were determined for all sample types fortified at 0.01 or 0.05 mg/kg. Recoveries for methomyl were generally acceptable, with only the occasional analysis outside the range 70-110%. A summary of the recovery data is presented in Table B.7.34.

Table B.7.34 Summary of the analytical procedural recovery data for milk and tissues from cows dosed with methomyl

Matrix	Fortification level (mg/kg)	Recovery range (%)	Average recovery (%) $\pm$ SD (n=)
Fat	0.01	82.9 - 86.9	85.1 $\pm$ 1.65 (3)
	0.05	96.8 - 75.8	86.3 (2)
Liver	0.01	98.4 - 116	106 $\pm$ 7.6 (4)
	0.05	85.4 - 95.5	89.6 $\pm$ 4.28 (3)
Muscle	0.01	81.5 - 100	93.6 $\pm$ 8.58 (3)
	0.05	82.4 - 89.6	86.0 (2)
Kidney	0.01	92.1 - 98.2	94.7 $\pm$ 2.57 (3)
	0.05	87.6 - 94.9	91.3 (2)
Whole milk	0.01	83.8 - 103	93.5 $\pm$ 6.62 (16)
	0.05	77.9 - 144	91.7 $\pm$ 15.6 (15)
Cream	0.01	93.6	n = 1
	0.05	94.0	n = 1
Skimmed milk	0.01	97.6	n = 1
	0.05	90.9	n = 1

Residues in all milk samples from the animals dosed at 34 and 86 mg/kg feed DM were individually all below the LOQ of 0.01 mg/kg. Analysis was not carried out on all the milk samples from the animals dosed at 8 mg/kg feed DM or because residues above 0.01 mg/kg were not anticipated.

Residues in all samples of muscle, liver, kidney and fat from all dose groups were also all individually below the LOQ of 0.01 mg/kg.

(Daun, R.J., 1995)

A poultry feeding study was not submitted. A feeding study is not required for the notified uses as the consumption of grape, cucumber, courgette, tomato and aubergine by animals is negligible.

**B.7.10 Residues in succeeding or rotational crops (IIA 6.6, IIIA 8.5)**

No data were submitted as significant residues in succeeding crops are unlikely (see Section B.7.1.4)

**B.7.11 Proposed pre-harvest intervals for envisaged uses or withholding periods, in the case of post harvest uses (Annex IIA 6.8, Annex IIIA 8.7)**

Current Good Agricultural Practice is summarised in Table 1.5.2. No additional information has been submitted.

**B.7.12 Community MRLs and MRLs in EU Member States (IIIA 12.2)**

The following MRLs were published in 2000 in Council Directive 2000/42/EC:

Table B.7.35 Community MRLs for methomyl

Commodity	EU MRL (mg/kg)
Table grape	0.05 (*)
Wine grape	1
Tomato	0.5
Aubergine	0.5
Cucurbits, edible peel	0.05 (*)

(\*) MRL set at LOQ.

**B.7.13 Proposed EU MRLs and justification for the acceptability of those MRLs (IIA 6.7, IIIA 8.6)**

Table B.7.36 Proposed EU MRLs

Commodity	Current EU MRL (mg/kg)	Proposed EU MRL following review under 91/414 (mg/kg)	Comments
Table grape	0.05	0.5	R(ber) = 0.410, R(max) = 0.465
Wine grape	1	0.5	
Cucumber	0.05	0.05	
Courgette	0.05	0.05	
Tomato	0.5	0.05	
Aubergine	0.5	0.05	

**B.7.14 Proposed EU Import tolerances and justification for the acceptability of those residues**

None

**B.7.15 Basis for differences, if any, in conclusions reached having regard to established or proposed CAC MRLs**

Not considered in this Draft Assessment Report.

**B.7.16 Estimates of potential and actual dietary exposure through diet and other means (IIA 6.9, IIIA 8.8)****B.7.16.1 Intakes by domestic animals.**

An assessment of the theoretical maximum daily intakes by domestic animals from the consumption of grape, cucumber, courgette, tomato and aubergine which may contain residues of methomyl has not been made as consumption of these commodities by animals is negligible.

**B.7.16.2 Intakes by humans****B.7.16.2.1 Long term intakes - National Estimate of Dietary Intake (NEDI)**

The UK long term dietary intakes (NEDIs) for residues of methomyl from the consumption of a number of crops individually have been calculated for adults, young people, toddlers, infants, vegetarians and elderly adults. In addition total dietary intakes (total NEDIs for all crops combined) were calculated using the Rees-Day model.

The following assumptions have been made:

- (i) upper range of normal (97.5th percentile) consumption of each individual crop which may have been treated.
- (ii) all produce eaten which may have been treated, has been treated and contains residues at the median level (STM<sub>R</sub>) found in the trials considered to support GAP as detailed below:

Commodity	STM <sub>R</sub> (mg/kg)
Grape	0.09
Cucumber	0.02
Courgette	0.02
Tomato	0.02
Aubergine	0.02

The proposed residue definition for risk assessment purposes is methomyl.

- (iii) there is no loss of residue during transport or storage of foods prior to consumption. The following processing factors have been used:

Commodity	Processed product	Processing factor
Grape	Wine	0.58*

\* mean of 4 processing studies.

The results of the chronic total dietary intake estimates (total NEDIs) based on the Rees-Day model are presented in Table B.7.37, together with NEDIs for individual commodities. The mean and 97.5th percentile consumption data are presented in Table B.7.38.

Based on chronic exposure estimates for long term dietary exposure all intakes are well below the proposed ADI of 0.005 mg/kg bw/day. The total NEDIs vary according to different consumer groups, although the values range from 2% (total NEDI of 0.00009 mg/kg bw/day for elderly (residential) consumer group) to 10% (total NEDI of

0.00048 mg/kg bw/day for toddler consumer group) of the proposed ADI. The long-term risks to consumers from consumption of these commodities are acceptable.

WARNING: This document forms part of an EC evaluation data package and should not be read in isolation. Registration must not be granted on the basis of this document.

Table B.7.37 UK Intakes (NEDIs) in mg/kg bw/day of residues of methomyl from treated foodstuffs [proposed ADI is 0.005 mg/kg bw/day]

		ADULT	INFANT	TODDLER	4-6 YEARS	7-10 YEARS	11-14 YEARS	15-18 YEARS	VEGETARIAN	ELDERLY (OWN HOME)	ELDERLY (RESIDENTIAL)
TOTAL		0.000305	0.000196	0.000479	0.000236	0.000272	0.000140	0.000179	0.000511	0.000340	0.000094
TOTAL as % ADI		6%	4%	10%	5%	5%	3%	4%	10%	7%	2%
Commodity	STMR (mg/kg) P										
Table grapes*	0.09	0.000109	0.000149	0.000423	0.000194	0.000233	0.000099	0.000055	0.000184	0.000119	0.000041
Wine grapes*	0.09 0.58	0.000186	0.000039	0.000028	0.000029	0.000010	0.000032	0.000113	0.000311	0.000213	0.000047
Tomatoes	0.02	0.000027	0.000037	0.000053	0.000038	0.000037	0.000022	0.000027	0.000035	0.000029	0.000027
Aubergines*	0.02	0.000006	L/C	0.000031	0.000015	0.000005	0.000011	0.000008	0.000012	0.000008	L/C
Cucumbers*	0.02	0.000009	0.000004	0.000048	0.000031	0.000021	0.000010	0.000009	0.000011	0.000009	0.000004
Courgettes*	0.02	0.000009	0.000030	0.000047	0.000026	0.000016	0.000008	0.000008	0.000011	0.000010	0.000009

Table B.7.38 Consumption in kg/day of relevant foods.

Mean values are population means, and 97.5<sup>th</sup> %ile are calculated on the basis of those consuming only.

Commodity	ADULT		INFANT		TODDLER		4-6 YEARS		7-10 YEARS		11-14 YEARS		15-18 YEARS		VEGETARIAN		ELDERLY (own home)		ELDERLY (residential)	
	mean	97.5%	mean	97.5%	mean	97.5%	mean	97.5%	mean	97.5%	mean	97.5%	mean	97.5%	mean	97.5%	mean	97.5%	mean	97.5%
Table grapes*	0.0020	0.0848	0.0002	0.0144	0.0036	0.0681	0.0028	0.0442	0.0038	0.0799	0.0018	0.0530	0.0008	0.0402	0.0051	0.1360	0.0030	0.0940	0.0007	0.0284
Wine grapes*	0.0286	0.2501	0.0001	0.0065	0.0003	0.0079	0.0003	0.0114	0.0003	0.0060	0.0007	0.0297	0.0052	0.1382	0.0333	0.3977	0.0174	0.2887	0.0019	0.0551
Tomatoes	0.0280	0.0932	0.0032	0.0159	0.0086	0.0382	0.0127	0.0394	0.0153	0.0569	0.0168	0.0518	0.0255	0.0851	0.0415	0.1180	0.0247	0.1030	0.0155	0.0825
Aubergines*	0.0004	0.0218	L/C	L/C	0.0001	0.0222	0.0001	0.0155	0.0001	0.0083	0.0001	0.0252	0.0002	0.0245	0.0019	0.0413	0.0002	0.0287	L/C	L/C
Cucumbers*	0.0043	0.0300	L/C	0.0019	0.0016	0.0351	0.0028	0.0316	0.0033	0.0322	0.0030	0.0249	0.0039	0.0294	0.0071	0.0364	0.0028	0.0336	0.0012	0.0110
Courgettes*	0.0009	0.0327	0.0001	0.0129	0.0002	0.0344	0.0002	0.0263	0.0002	0.0242	0.0001	0.0201	0.0003	0.0243	0.0032	0.0371	0.0005	0.0365	0.0003	0.0276

\* <60 consumers in one or more groups.

L/C Low consumption (<0.1 g/day) or low number of consumers (<4).

Calculations have also been carried out using the WHO Standard European diet and are shown in Table B.7.39

Table B.7.39 Intake of methomyl from treated foodstuffs.

crop	intake	
	crop/food (g/person/day)	methomyl (mg/kg bw/day)
Grapes	13.8	0.000116
Tomatoes*	66.0	0.000019
Tomatoes, fresh	38.2	0.000011
Eggplants	2.3	0.000001
Cucumbers & gherkins	9.0	0.000003
Fruiting veg., cucurbits	38.5	0.000011

\*= tomato fresh + (tomato juice x 1.06) + (tomato paste x 6.4) + tomato peeled.

All intakes are well below the proposed ADI of 0.005 mg/kg bw/day.

#### B.7.16.2.2 Short term intakes - National Estimate of Short Term Intake (NESTI)

The NESTIs for residues of methomyl from the consumption of a number of crops have been calculated for adults and toddlers. The following assumptions have been made:

- upper range of normal (97.5th percentile) consumption of each individual crop which may have been treated.
- all produce eaten which may have been treated, has been treated and contains residues at the highest residue level (HR) found in the trials considered to support GAP as detailed below:

Commodity	Highest residue (mg/kg)
Grape	0.59
Cucumber	0.02
Courgette	0.02
Tomato	0.02
Aubergine	0.02

The proposed residue definition for risk assessment purposes is methomyl.



(iii) there is no loss of residue during transport or storage of foods prior to consumption.

The following processing factors have been used:

Commodity	Processed product	Processing factor
Grape	Wine	0.58*

\* mean of 4 processing studies.

The relevant consumption data and acute intake estimates are presented in Table B.7.40.

Table B.7.40 Intakes (NESTIs) of residues of methomyl from treated foodstuffs [proposed ARfD is 0.005 mg/kg bw/day].

commodity	HR (mg/kg)	V	P	Adult			Toddler			Infant			4-6 year old child		
				NESTI (mg/kg bw/day)	% ARfD	acute consumption (kg/day)	NESTI (mg/kg bw/day)	% ARfD	acute consumption (kg/day)	NESTI (mg/kg bw/day)	% ARfD	acute consumption (kg/day)	NESTI (mg/kg bw/day)	% ARfD	acute consumption (kg/day)
*Wine	0.090	1		0.0010	19.9	0.774	0.0003	5.2	0.042	0.0004	8.6	0.042	0.0003	7.0	0.080
Table Grapes	0.590	5		<b>0.0133</b>	<b>267.0</b>	0.317	<b>0.0360</b>	<b>720.2</b>	0.177	<b>0.0170</b>	<b>339.1</b>	0.050	<b>0.0297</b>	<b>594.9</b>	0.207
Tomatoes	0.020	7		0.0002	4.3	0.245	0.0008	16.6	0.091	0.0010	19.3	0.060	0.0006	12.4	0.127
Aubergine	0.020	5		0.0002	3.7	0.130	0.0004	8.6	0.062	0.0000	0.0	0.000	0.0005	10.0	0.103
Cucumber	0.020	5		0.0001	2.5	0.086	0.0006	11.8	0.086	0.0001	3.0	0.013	0.0005	9.4	0.096
Courgettes	0.020	7		0.0002	4.8	0.165	0.0009	18.6	0.096	0.0006	12.7	0.039	0.0008	16.0	0.136

commodity	HR (mg/kg)	V	P	7-10 year old child			11-14 year old child			15-18 year old child			Vegetarian		
				NESTI (mg/kg bw/day)	% ARfD	acute consumption (kg/day)	NESTI (mg/kg bw/day)	% ARfD	acute consumption (kg/day)	NESTI (mg/kg bw/day)	% ARfD	acute consumption (kg/day)	NESTI (mg/kg bw/day)	% ARfD	acute consumption (kg/day)
*Wine	0.090	1		0.0001	2.1	0.035	0.0004	7.8	0.208	0.0010	20.9	0.742	0.0012	23.6	0.875
Table Grapes	0.590	5		<b>0.0274</b>	<b>547.4</b>	0.287	<b>0.0214</b>	<b>427.5</b>	0.348	<b>0.0107</b>	<b>213.4</b>	0.231	<b>0.0180</b>	<b>360.5</b>	0.408
Tomatoes	0.020	7		0.0004	8.9	0.174	0.0003	5.6	0.168	0.0002	4.8	0.251	0.0003	5.4	0.397
Aubergine	0.020	5		0.0002	3.7	0.058	0.0002	5.0	0.119	0.0002	3.2	0.102	0.0004	7.1	0.236
Cucumber	0.020	5		0.0004	7.1	0.100	0.0002	3.9	0.093	0.0002	3.7	0.117	0.0002	3.5	0.115
Courgettes	0.020	7		0.0005	10.3	0.115	0.0002	4.6	0.079	0.0002	3.9	0.088	0.0003	5.4	0.211

commodity	HR (mg/kg)	V	P	Elderly – own home			Elderly - residential		
				NESTI (mg/kg bw/day)	% ARfD	acute consum ption (kg/day)	NESTI (mg/kg bw/day)	% ARfD	acute consu- mption (kg/day)
*Wine	0.090	1		0.0007	14.5	0.569	0.0002	3.7	0.127
Table Grapes	0.590	5		<b>0.0067</b>	<b>133.0</b>	0.160	0.0048	95.0	0.099
Tomatoes	0.020	7		0.0002	4.0	0.200	0.0002	4.7	0.218
Aubergine	0.020	5		0.0001	2.7	0.095	0.0000	0.0	0.000
Cucumber	0.020	5		0.0001	2.6	0.092	0.0001	1.1	0.035
Courgettes	0.020	7		0.0002	4.5	0.117	0.0003	5.0	0.111

Based on acute exposure estimates for short term dietary exposure, intakes for the consumption of cucumber, courgette, tomato and aubergine are well below the proposed ARfD of 0.005 mg/kg bw/day. The short term risks to consumers from consumption of these commodities are acceptable.

The intakes for the consumption of table grapes exceed the proposed ARfD for the majority of consumer groups (shown in bold in Table B.7.40) using a variability factor of 5.

The notifier submitted probabilistic modelling data for adults and toddlers, performed with the DEEM-UK™ model using residue data generated from the supervised trials.

A residue value of 0.01 mg/kg (half the LOQ) was used for tomato, aubergine, cucumber and courgette risk assessments. The residue distribution values for grapes were generated by randomly applying a variability factor obtained from a lognormal distribution of potential variability factors to a randomly selected residue trial result. The lognormal distribution for the variability factors had a minimum value of 1, a maximum of 7 and a mean of 4. 10 000 individual residue values for grapes were generated for use in the probabilistic model. Analysis was conducted with the model using 1000 iterations for assessments that did not include grapes and with 5000 iterations for those assessments that included grapes. All exposure values were evaluated at the 99.9<sup>th</sup> percentile. Processing factors were applied using real data where available (e.g. for grape juice and wine production) and using default factors when no data were available (e.g. for tomato juice). Data were generated on a 'consumer' and 'per capita' basis and also for the assumptions that 100% and 50% of the crop were treated. Data were generated for the consumption of individual crops and also for the consumption of any or all of the crops combined. The data generated on a consumer basis are shown in Table B.7.41.

Table B.7.41 Acute intakes of residues of methomyl from treated foodstuffs using the DEEM UK<sup>TM</sup> probabilistic risk assessment model.

POPULATION	CROP	% CROP TREATED	INTAKE (mg/kg bw/day)	%ARfD*
Adults	All**	100%	0.002700	54.0
		50%	0.001877	37.5
Toddlers	All**	100%	0.009994	<b>199.9</b>
		50%	0.007823	156.5
Adults	Grapes only	100%	0.003766	75.3
		50%	0.002794	55.9
Toddlers	Grapes only	100%	0.011241	<b>224.8</b>
		50%	0.005004	180.1
Adults	Tomato & aubergine only	100%	0.000422	8.4
		50%	0.000352	7.0
Toddlers	Tomato & aubergine only	100%	0.000849	17.0
		50%	0.000769	15.4
Adults	Cucumber & courgette only	100%	0.000200	4.0
		50%	0.000189	3.8
Toddlers	Cucumber & courgette only	100%	0.001043	20.9

\*These data were originally calculated using an ARfD of 0.01 mg/kg bw/day but have been corrected in the table to reflect the proposed ARfD of 0.005 mg/kg bw/day.

\*\* Total intake for the consumption of any or all of the crops.

The intakes for the consumption of cucumber, courgette, tomato and aubergine are below the proposed ARfD of 0.005 mg/kg bw/day. A value of 0.01 mg/kg was used for exposure estimates. Using a value of 0.02 mg/kg (LOQ) will not lead to exceedance of the proposed ARfD. The short term risks to consumers from consumption of these commodities are acceptable.

The intakes for consumption of grapes only for adults are below the proposed ARfD but are above the proposed ARfD for toddlers when 100% of the crop is treated. The proposed ARfD is still exceeded for toddlers when only 50% of the crop is treated. The 50% value was an arbitrary figure chosen by the notifier to demonstrate that the proportion of crop treated will affect the risk to consumers, but since only intakes due to the consumption of treated crops are of relevance to the risk assessment the data are not discussed further. The grapes residues data used to generate the intakes were taken from a smaller data set than those considered acceptable by the RMS.

The data for total intakes due to the consumption of any or all crops are not considered relevant as the introduction of other variables, such as consumers who did not eat grapes but ate other commodities, may lead to lower intake values. This would in turn give rise to an underestimate of the risks to consumers.

The notifier suggests that more realism could be introduced into the grape residue distribution by accounting for the probability of residues being reduced by washing prior

to consumption. The assumption that residues are reduced by washing cannot be made without supporting data.

Given the concerns outlined above, and that the use on grapes gives rise to an exceedance of the proposed ARfD, inclusion on Annex I for the use on grapes cannot be recommended.

Inclusion on Annex I based on the uses on tomato, aubergine, cucumber and courgette can be recommended.

#### B.7.17 References relied on

Author(s)	Annex No., Reference No.	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	EU Data Protec- tion Claimed (Y/N)	Owner
Dietrich, R.F., Charlton, R.R., Ryan, D.L., McAler, N.C., Bookhart, S.W.	IIA, 6.2.1./01	1995	The metabolism of <sup>14</sup> C-methomyl in the lactating goat DuPont Experimental Station AMR 2701-93 (2 volumes) GLP: Yes Published: No	Y	DuPont
Djanegara, T.K.S., Ryan, D.L.	IIA, 6.2.2./01	1994	Metabolism of methomyl in laying hens. DuPont Experimental Station, Battelle (Ohio) AMR 2217-91 GLP: Yes Published: No	Y	DuPont
Françon, B., Larcinese, J.P.	IIA, 6.3.1.2./01	1999	Combined decline and magnitude of residues of methomyl insecticide in/on solanacea vegetables (tomatoes) grown in southern Europe following treatment with Lannate® 20L and/or Lannate® 25WP - season 1997 - (analysis by HPLC) Battelle Europe-Centre de Recherche de Geneve AMR 4507-97 GLP: Yes Published: No	Y	DuPont

Author(s)	Annex No., Reference No.	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	EU Data Protection Claimed (Y/N)	Owner
Françon, B., Larcinese, J.P.	IIA, 6.3.1.3./01	1999	Combined decline and magnitude of residues of methomyl insecticide in/on cucurbits (cucumbers and courgettes) grown in southern Europe following treatment with Lannate® 20L and/or Lannate® 25WP - season 1997 - (analysis by HPLC) Battelle Europe-Centre de Recherche de Geneve AMR 4511-97 GLP: Yes Published: No	Y	DuPont
Harvey, J.	IIA, 6.1.1.1./01	1967	Metabolism of S-methyl N-[(methylcarbamoyloxy]-thioacetimidate in the tobacco plant DuPont Experimental Station ML/ME 1 GLP: No Published: No	Y	DuPont
Harvey, J.	IIA, 6.1.1.2./03	1970	Metabolism of methomyl in corn and cabbage: effect of $\beta$ -glucosidase on the polar fraction DuPont Experimental Station ML/ME 5 GLP: No Published: No	Y	DuPont
Harvey, J.	IIA, 6.1.1.3./03	1970	Metabolism of methomyl in corn and cabbage: effect of $\beta$ -glucosidase on the polar fraction DuPont Experimental Station ML/ME 5 GLP: No Published: No	Y	DuPont
Harvey, J. Jr., Buchanan, J.B.	IIA, 6.1.1.1./02	1968	Absence of S-oxide and S,S-dioxide as potential metabolites of methomyl in soil, tobacco and rats DuPont Experimental Station ML/ME 25 GLP: No Published: No	Y	DuPont
Harvey, J., Reiser, R.W.	IIA, 6.1.1.2./04	1973	Metabolism of methomyl in tobacco, corn and cabbage DuPont Experimental Station ML/ME 48 GLP: No Published: Yes	Y	DuPont

Author(s)	Annex No., Reference No.	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	EU Data Protection Claimed (Y/N)	Owner
Harvey, J., Reiser, R.W.	IIA, 6.1.1.3./02	1968	Metabolism of methomyl in corn and cabbage: characterisation of the polar and non-polar fractions DuPont Experimental Station ML/ME 4 GLP: No Published: No	Y	DuPont
Harvey, J., Reiser, R.W.	IIA, 6.1.1.3./04	1973	Metabolism of methomyl in tobacco, corn and cabbage DuPont Experimental Station ML/ME 48 GLP: No Published: Yes	Y	DuPont
Harvey, J., Yates, R.A.	IIA, 6.1.1.3./01	1968b	Metabolism of methomyl in the corn plant DuPont Experimental Station. ML/ME 2 GLP: No Published: No	Y	DuPont
Harvey, J., Reiser, R.W.	IIA, 6.1.1.1./03	1973	Metabolism of methomyl in tobacco, corn and cabbage DuPont Experimental Station ML/ME 48 GLP: No Published: Yes	Y	DuPont
Harvey, J., Reiser, R.W.	IIA, 6.1.1.2./02	1968	Metabolism of methomyl in corn and cabbage: characterisation of the polar and non-polar fractions DuPont Experimental Station ML/ME 4 GLP: No Published: No	Y	DuPont
Harvey, J., Yates, R.A.	IIA, 6.1.1.2./01	1968a	Metabolism of methomyl in cabbage DuPont Experimental Station ML/ME 3 GLP: No Published: No	Y	DuPont
Kennedy, C.M., Bentley, K. S.	various	2004	Response to PSD regarding the plant residue definition for risk assessments 5 March 2004.		DuPont
Kennedy, C.M., Devine, P.G.	IIA, 6.0.1.3./01	1993	Freezer storage stability of methomyl in broccoli DuPont Experimental Station, Morse Laboratories, Inc. AMR 1765-90 GLP: Yes Published: No	Y	DuPont



Author(s)	Annex No., Reference No.	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	EU Data Protection Claimed (Y/N)	Owner
Kennedy, C.M., Devine, P.G.	IIA, 6.0.1.4./01	1993	Freezer storage stability of methomyl in lettuce DuPont Experimental Station, Morse Laboratories, Inc. AMR 1764-90 GLP: Yes Published: No	Y	DuPont
Kennedy, C.M., Tomic, D.M.	IIA, 6.0.1.1./01	1993	Freezer storage stability study of methomyl in grapes DuPont Experimental Station, Morse Laboratories, Inc. AMR 1769-90 GLP: Yes Published: No	Y	DuPont
Milby, K.H.	IIA, 6.0.1.5./01	2000	Stability of methomyl residues in frozen analytical samples of bean seed, potato tubers, and peanut nutmeats following application of Lannate® SP insecticide DuPont Experimental Station, Morse Laboratories, Inc. AMR 3741-96 GLP: Yes Published: No	Y	DuPont
Nathan III, E.C., Dubey, L., Steiner, C.	IIA, 6.3.1.1./02	2002	Combined decline and magnitude of residues of methomyl in wine and table grapes (berries and small fruit) following applications of DPX-X1179 20SL – northern/southern Europe, season 2001 Battelle Europe-Centre de Recherche de Geneve DuPont-5831 GLP: Yes Published: No	Y	DuPont
Nathan III, E.C., Dubey, L., Steiner, C., Mattou, H.	IIA, 6.3.1.1./01	2001	Combined decline and magnitude of residues of methomyl in wine and table grapes (berries and small fruit) following applications of DPX-X1179 20SL and DPX-X1179 25WP formulations - Europe, season 2000 Battelle Europe-Centre de Recherche de Geneve DuPont-4356 GLP: Yes Published: No	Y	DuPont

## Methomyl – Volume 3, Annex B: Residues

April 2004

Author(s)	Annex No., Reference No.	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	EU Data Protection Claimed (Y/N)	Owner
Nathan III, E.C., Dubey, L., Steiner, C., Matou, H.	IIA, 6.5.2./01	2001	Residues of methomyl in processed fractions of wine grapes (berries and small fruit) following applications of Methomyl 20SL formulation - Europe, season 2000 Battelle Europe-Centre de Recherche de Geneve DuPont-4452 Part A GLP: Yes Published: No	Y	DuPont
Nathan III, E.C., Dubey, L., Steiner, C., Mattou, H.	IIA, 6.0.1.1./02	2002	Residues of methomyl in processed fractions of wine grapes (berries and small fruit) following applications of Methomyl 20SL formulation - Europe, season 2000 Battelle Europe-Centre de Recherche de Geneve DuPont-4452, Part B GLP: Yes Published: No	Y	DuPont
Pedersen, C.T.	IIA, 6.5.1./01	2001	Hydrolysis of [1- <sup>14</sup> C]methomyl (DPA-X1179) technical in pH 4, 5, and 6 buffer solutions at high temperatures DuPont Crop Protection, Stine-Haskell Research Center DuPont-5772 GLP: Yes Published: No	Y	DuPont
Rühl, J.C., Devine, P.G.	IIA, 6.0.1.6./01	1994	Freezer storage stability in corn DuPont Experimental Station, Morse Laboratories, Inc. AMR 1770-90 GLP: Yes Published: No	Y	DuPont
Ryan, D.L., McMillan, J.A., Young, G.A.	IIA, 6.1.1a./01	2003	The metabolism of <sup>14</sup> C-methomyl in grapes DuPont Stine Haskell Research Center DuPont-6589 GLP: Yes Published: No	Y	DuPont

Author(s)	Annex No., Reference No.	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	EU Data Protection Claimed (Y/N)	Owner
Weidenauer, M., Françon, B., Larcinese, J.P.	IIA, 6.3.1.2./02	1998	Combined decline and magnitude of residues of methomyl insecticide in/on solanacea vegetables (tomatoes and peppers) grown in southern Europe following treatment with Lannate® 20L and/or Lannate® 25WP - season 1996 - (analysis by HPLC) Battelle Europe-Centre de Recherche de Geneve AMR 3999-96 GLP: Yes Published: No	Y	DuPont
Weidenauer, M., Françon, B., Larcinese, J.P.	IIA, 6.3.1.3./02	1998	Combined decline and magnitude of residues of methomyl insecticide in/on cucurbits (cucumbers and courgettes) grown in southern Europe following treatment with Lannate® 20L and/or Lannate® 25WP - season 1996 - (analysis by HPLC) Battelle Europe-Centre de Recherche de Geneve AMR 3977-96 GLP: Yes Published: No	Y	DuPont

### Plant Protection Product – Methomyl 20 SL

All data to address residues aspects were submitted under Annex IIA of the dossier. No residues data were submitted in the Annex IIIA dossier.

#### B.7.18 References used in assessment of data

1. J Gregory, K Foster, H Tyler, M Wiseman. Dietary and Nutritional Survey of British Adults, HMSO, 1990.
2. J Gregory, S Lowe, C J Bates, A Prentice, L V Jackson, G Smithers, R Wenlock & M Farron, National Diet and Nutrition Survey: Young people aged 4 to 18 years, Volume 1: Report of the diet and nutrition survey, The Stationery Office, 2000

3. J R Gregory, D L Collins, P S W Davies, J M Hughes & P C Clarke. National Diet and Nutrition Survey; Children Aged 11/2 - 41/2 Years. Volume 1: Report of the diet and nutrition survey, HMSO, 1995
4. Mills, A. & Tyler, H. Food and Nutrient Intakes of British Infants Aged 6-12 Months, HMSO, 1992
5. J G Steele, A Sheiham, W Marcenes & A W G Walls. National Diet and Nutrition Survey: People aged 65 years and over. Volume 2: Report of the Oral Health survey, The Stationery Office, 1998
- 6a. Ministry of Agriculture, Fisheries and Food, Research & Development and Surveillance Report: 181 (October 1996). Dietary Survey of Vegetarians: Final Technical Report.
- 6b. Ministry of Agriculture, Fisheries and Food, Research & Development and Surveillance Report: 303 (June 1997). Dietary Survey of Vegetarians: Tables of Questionnaire Results.
- 6c. Ministry of Agriculture, Fisheries and Food, Research & Development and Surveillance Report: 261 (July 1997). Dietary Survey of Vegetarians: Analysis of the Questionnaire Results.
- 6d. Ministry of Agriculture, Fisheries and Food, Research & Development and Surveillance Report: (March 1999). Vegetarians Dietary Survey: Technical Report on Weighed Intake Diary Data.