FEDERAL INSTITUTE FOR RISK ASSESSMENT

Bundesinstitut für Risikobewertung

Developmental Neurotoxicity

EU regulatory perspective with special focus on pesticides

Andrea Gall and Roland Solecki

German Federal Institute for Risk Assessment, Berlin

Regulatory DNT Approaches for Pesticides

Legal framework

Plant Protection Products

Regulation (EC) 1107/2009

Data requirements legally binding (Reg. (EU) 283/2013)

- ***5.6.2. Developmental toxicity studies***
- Developmental toxicity studies with other relevant data and information on the active substance, shall be sufficient to permit the assessment of effects on embryonic and foetal development, following repeated exposure to the active substance…
- When indicated by observations in other studies or the mode of action of the test substance, supplementary studies or information may be required to provide information on the postnatal manifestation of effects such as **developmental neurotoxicity**.
- **5.7.1.** ***Neurotoxicity studies in rodents***
- Neurotoxicity studies in rodents shall provide sufficient data to evaluate the potential neurotoxicity of the active substance (neurobehavioural and neuropathological effects) after single and repeated exposure.

Regulatory DNT Approaches for Pesticides
Legal framework

Biocidal Products in Europe

Regulation (EC) 528/2012

Data requirements legally binding (A.-II Reg. (EU) 528/2012)

- ***Article 19 Conditions for granting an authorisation***
  …the simplified authorisation procedure acc. Article 25
  A biocidal product shall not be authorised where:…
  it has **developmental neurotoxic** or immunotoxic **effects**.

ANNEX II INFORMATION REQUIREMENTS FOR AS

- **8.10.1. Pre-natal developmental toxicity study (Core Data Set)**
- **8.10.3. Further pre-natal developmental toxicity study (Additional Data Set)**
- **8.13.2. Neurotoxicity including DNT (Additional Data Set)**
  if there is any evidence knowledge of the mechanism of action or from repeat dose studies that the a.s. may have neurotoxic or
  developmental neurotoxic properties
  then additional information or specific studies will be required.

Regulatory DNT Approaches for Pesticides

Legal framework

Regulation (EC) No. 1107/2009 (PPP):

„An active substance,…, shall only be approved [...]

- if it is not or has not to be classified, in accordance with the provisions of Regulation (EC) No 1272/2008, as toxic for **reproduction category 1A or 1B** [...]“

Regulation (EU) No. 528/2012 (BP):

“ the following active substances shall not be approved: […]

- active substances which have been classified in accordance with Regulation (EC) No 1272/2008 as, or which meet the criteria to be classified
  as toxic for **reproduction category 1A or 1B**

unless…negligible exposure/risk

Regulatory DNT Approaches for Pesticides

Case studies, how DNT data usually handled

Risk Assessment and Setting of Reference Values; Acute Reference Dose (ARfD) and Acceptable Daily Intake (ADI)

- **Data availability**:
  - Developmental toxicity studies
  - Acute and repeated neurotoxicity
  - Developmental neurotoxicity study (not mandatory in Europe)
- **Relevant effects:**
  - Embryo-/fetotoxicity (e.g. death, growth retardation)
  - Structural defects (malformations, variations)
  - Neurological effects (structural, neurobiochemical, behaviour)
- **Conclusions**:
  - Developmental, multigeneration, acute and short-term neurotoxicity studies, but also developmental (neurotoxic) effects relevant for ARfD and ADI.
  - Consider length of the critical window, kinetics, mechanism.
  - Indications from in vitro DNT Testing considered to be supportive

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Case studies, how DNT data usually handled

2002 - Report of the Joint Meeting of the

FAO Panel of Experts on Pesticide Residues

Results of DNT studies summarized in a EPA working paper were reviewed to examine the impact of DNT studies on ARfD and ADI:

- DNT studies on 14 pesticides evaluated by the US EPA were reviewed.
- Both generic and chemical-specific experimental DNT study designs were considered.
- Toxicity end-points of each DNT study and four related studies compared (developmental, multigeneration, acute/short-term neurotoxicity studies)
- The comparison showed that, in general, the majority of DNT studies did not identify significantly lower NOAELs and LOAELs compared to those of the other four related studies.
- With OP pesticides, functional and pathological effects in DNT studies not at lower doses than those at which cholinesterase inhibition was observed.

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Case studies, how DNT data usually handled

Table 1. Pesticides grouped by study type used for ARfD derivation in

Studies used for ARfD derivation	Number of substances	Percentage (%)
ARfD based on special studies	8	4.0
ARfD based on acute neurotoxicity studies in rats	20	10.1
ARfD based on repeated-dose studies in rats or dogs	16	8.1
ARfD based on multi-generation reproduction studies in rats	3	1.5
ARfD based on developmental toxicity studies in rats or rabbits	53	26.8
ARfD based on DNT studies in rats	2	1.0
ARfD based on human data	1	0.5
No ARfD was considered necessary	95	48.0

Critical Reviews in Toxicology, 2010; 40(1): 24–34

informa healthcare

REVIEW ARTICLE

A retrospective analysis of Acute Reference Doses for pesticides evaluated in the European Union

Roland Solecki, Tomas Moeller, Michael Herrmann, and Bernd Stein

Department of Chemical Safety, Federal Institute for Risk Assessment, Berlin, Germany

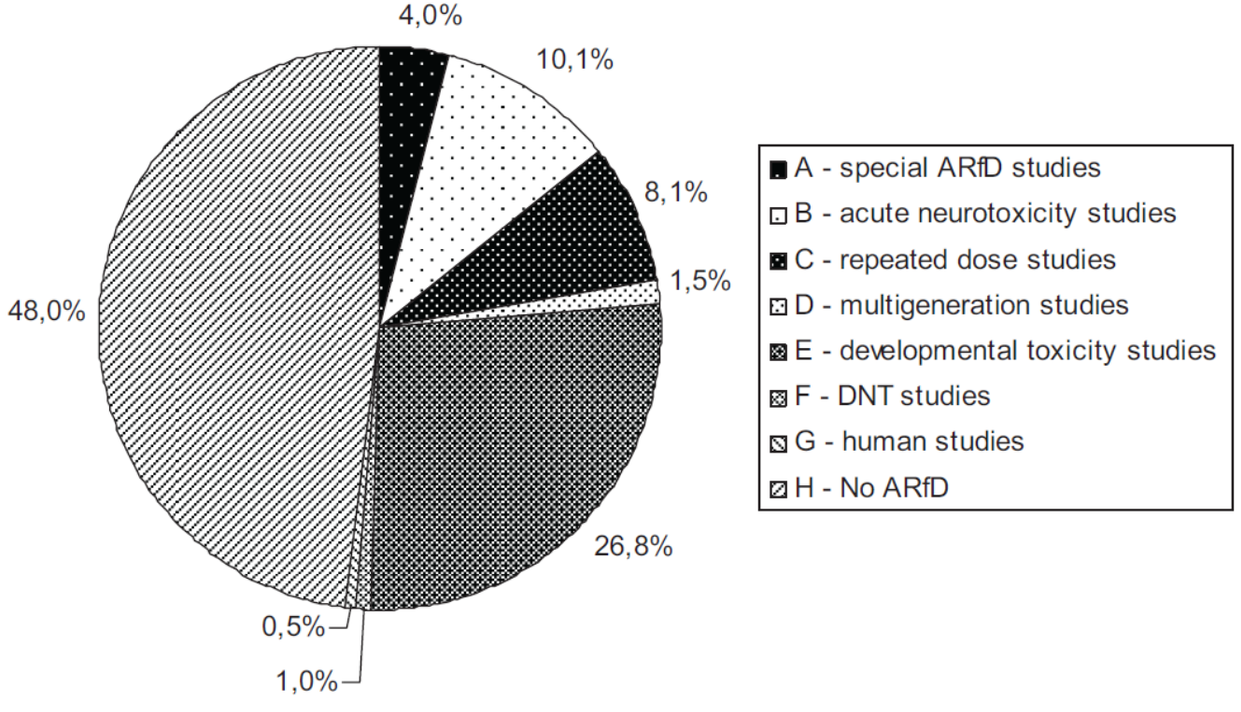

Figure 1. ARfD derivation in the EU pesticide evaluation program.

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Case studies, how DNT data usually handled

HSE

Supporting Publications 2013:EN-413

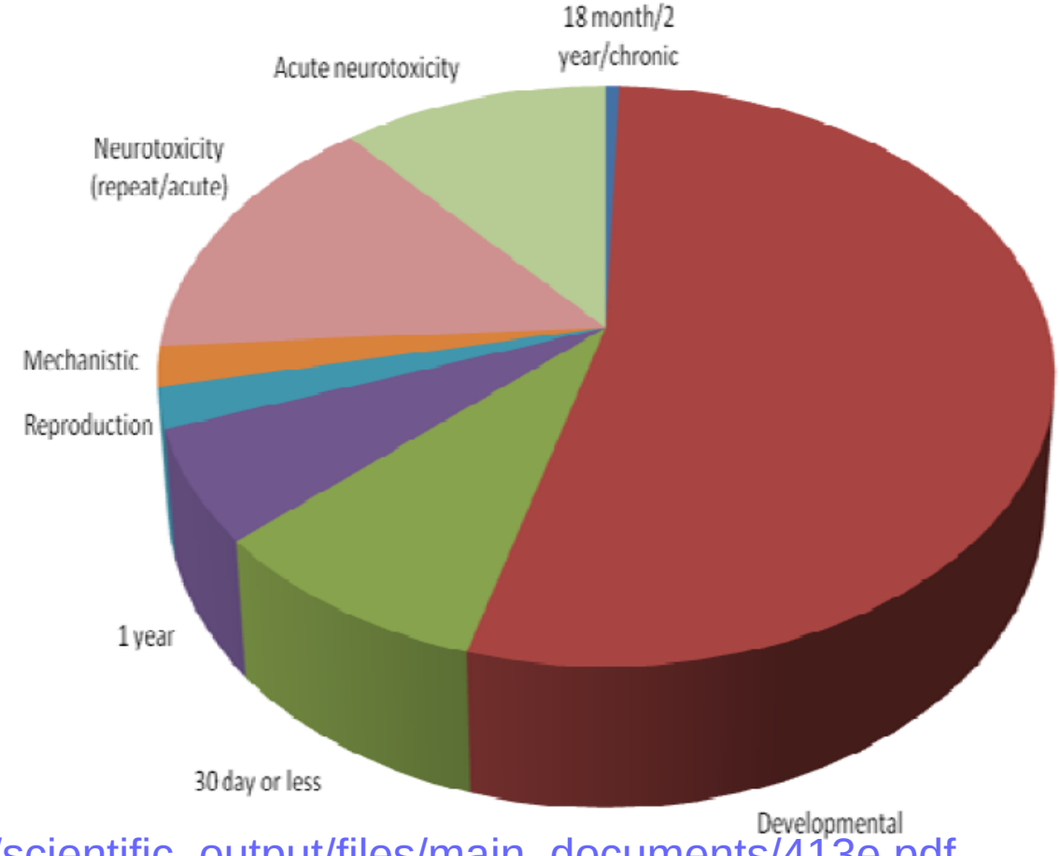
EXTERNAL SCIENTIFIC REPORT

Investigation of the state of the art on identification of appropriate reference points for the derivation of health-based guidance values (ADI, AOEL and AAOEL) for pesticides and on the derivation of uncertainty factors to be used in human risk assessment[1]

Chemicals Regulation Directorate, Health & Safety Executive, UK

Frequency of main study types used to set ARfD

18 month / 2 year / chronic	1%
1 year	8%
90 day	8%
2 - 30 day	10%
Mechanistic	3%
Developmental	58%
Reproduction	3%
Neurotoxicity (repeat /acute)	19%
Acute neurotoxicity	16%



http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/413e.pdf

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Evaluation of DNT studies on pesticides

The impact of DNT studies on ARfD and ADI setting:

- Available DNT studies on pesticidal active substances in plant protection products were analysed to examine.
- DNT studies on **35 substances** **out of 485 pesticidal active substances**, currently approved in the EU were reviewed:
  - **21 insecticides, 7 fungicides, 6 herbicides, 1 acaricide**
  - **19 positive tested substances** out of the 35 available screening and/or DNT studies were considered:
  - **15 insecticides, 2 herbicides & 2 fungicides**
  - 18 tested substances revealed evidence of DNT as well as neurotoxicity
  - 1 DNT positive tested fungicide did not reveal any evidence of neurotoxicity in adult rats (acute, FOB in 28-d, DNT)

Regulatory DNT Approaches for Pesticides

Evaluation of DNT studies on pesticides

The impact of DNT studies on ARfD and ADI setting:

- Reference values for the 19 positive tested pesticidal active substances are currently based on:
- **2 substances on DNT studies**
- 1 substance on a DNT study, but on developmental toxic effects
- 1 substance on an in-vivo Comparative Cholinesterase Assay
- 8 substances (ARfD) on developmental toxicity studies (rat, rabbit)
- 7 substances (ARfD) on neurotoxicity studies (acute, repeated)

- DNT is covered by both, ADI & ARfD in 15/19 studies
- DNT is covered by ADI, only in 2/19 studies (ARfD has to be re-evaluated)
- DNT not covered by ADI & ARfD in 1/19 studies (currently under discussion)
- 1 additional (positive) DNT has not yet been peer reviewed at EU level

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Case studies, how DNT data usually handled

Neonicotinoid insecticides under discussion:

Acetamiprid,

current EU ADI 0.07 mg/kg bw based on 2-yr & 2-generation, rat;
AOEL 0.124 mg/kg bw/d based on 90-d, rat;
ARfD 0.1 mg/kg bw based on acute neurotoxicity, rat.

PPR Panel (2013) considered that the current reference values may not be protective enough for possible DNT and ,
recommends a more conservative NOAEL of 2.5 mg/kg bw/d
[note: based on *supplementary DNT study, supported by in-vitro data*]
ADI, ARfD & AOEL**, which all should be set at **0.025 mg/kg bw/d
New and more reliable DNT data are required, the point of departure can be revised.

Imidacloprid,

current EU ADI 0.06 mg/kg bw based on 2-yr, rat;
AOEL & ARfD 0.08 mg/kg bw/d based on 28-d & 90-d, dog,
supported by subchronic neurotoxicity, rat.

PPR Panel (2013): *current ARfD and AOEL not be protective enough for potential DNT*
recommends to conservatively lower these reference values to the same level as ADI 0.06 mg/kg bw'

Regulatory DNT Approaches for Pesticides

Case studies, how DNT data usually handled

Glufosinate-ammonium

ADI & ARfD: **0.021 mg/kg bw** based on the NOAEL of 6.3 mg/kg bw/d (developmental toxicity, rabbit) and application of a safety factor of 300.

ARfD is considered to be adequately protective for any reproductive and developmental neurotoxic effects:

Rat		**NOAEL**	**LOAEL**
Developmental toxicity study	Maternal toxicity	10 mg/kg bw/d	50 mg/kg bw/d
	Embryo/foetal toxicity	10 mg/kg bw/d	50 mg/kg bw/d
DNT study	Maternal toxicity	69 mg/kg bw/d	292 mg/kg bw/d
	DNT	14 mg/kg bw/d	69 mg/kg bw/d
Rabbit			
Developmental toxicity study	Maternal toxicity	6.3 mg/kg bw/d	20 mg/kg bw/d
	Embryo/foetal toxicity	6.3 mg/kg bw/d	20 mg/kg bw/d

Regulatory DNT Approaches for Pesticides

Case studies, how DNT data usually handled

Chlorpyrifos

EU (2005), ARfD of 0.1 mg/kg bw based on:

- Acute neurotoxicity study in rats, NOAEL of 10 mg/kg bw
- Single oral gavage dose, no inhibition of brain AChE at 10 mg/kg bw
- Scientifically/ethically valid study in volunteers, NOEL was 1 mg/kg bw

As a part of the re-registration for Chlorpyrifos, the USEPA called for an **Comparative Cholinesterase Assay (CCA) study in rats** to determine, if age-related sensitivities to ChE inhibition exist:

- NOAELs after acute/repeated exposure are the same in pups and adults
- No clear evidence, that pups are more sensitive

New available toxicological data lowered the reference value (2014):

ARfD of 0.005 mg/kg bw, based on acute CCA, rat

Regulatory DNT Approaches for Pesticides

Summary/Take Home Message

- Data requirements for the evaluation of plant protection products and biocidal products in the EU are legally binding.
- DNT testing is not mandatory, but may be required for pesticidal active substances, if indications for DNT effects from other mandatory studies.
- DNT studies on **35 out of 485 pesticidal AS** approved in EU are available, 19 revealed positive in vivo evidence of DNT, majority not tested for DNT.
- Reference values for 2 positive tested substances are currently based on DNT studies, 2 are currently under discussion.
- In positive tested substances, DNT effects are covered by risk assessment including both ADI and ARfD, or at least the ADI.
- DNT testing can be considered not sufficient, although the majority of risk assessments can be considered protective for positive in vivo DNT effects.
- For regulatory purposes, identification of DNT compounds by an adequate *in vitro* testing battery is considered essential as a first screening step.

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Thank you for your attention

Andrea Gall and Roland Solecki

Federal Institute for Risk Assessment

Max-Dohrn-Str. 8-10 ● D-10589 Berlin

Tel. +49 30 - 184 12 - 3232

Roland.solecki@bfr.bund.de ● www.bfr.bund.de