NEUROSOME

Exploring The Neurological Exposome

Neurotoxicity Testing For Pesticides Mixtures:

Research Applications And Regulations Under RIrs Concept

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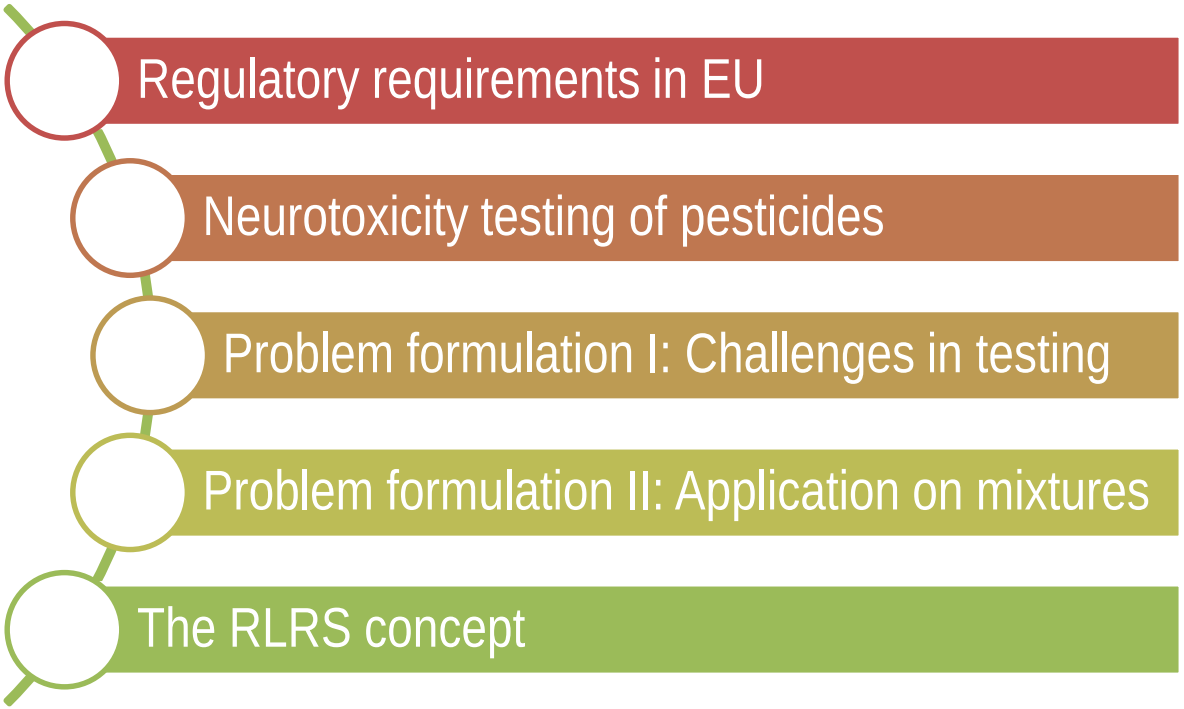
This project has received funding from the European Union's H2020 Framework Programme under grant agreement No - GA - 766251

Presentation Outline



Regulatory requirements

COMMISSION REGULATION (EU) No 283/2013

setting out the data requirements for active substances, in accordance with Regulation (EC) No 1107/2009

5.7. Neurotoxicity studies
- 5.7.1. Neurotoxicity studies in rodents
- 5.7.2. Delayed polyneuropathy studies

5.6.2. Developmental toxicity studies

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.

Regulatory requirements

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.

Circumstances in which required

- *Active substances with structures that are similar or related to those capable of inducing neurotoxicity*
- *Active substances which induce specific indications of potential neurotoxicity, neurological signs or neuropathological lesions in toxicity studies at dose levels not associated with marked general toxicity.*
- *Substances with a neurotoxic mode of pesticidal action.*
- *Neurotoxicity investigations in routine toxicology studies.*

Regulatory requirements

5.7.2. Delayed polyneuropathy studies

Delayed polyneuropathy studies shall provide sufficient data to evaluate if the active substance may provoke delayed polyneuropathy after ***acute and repeated exposure.***

A repeated exposure study may be waived unless there are indications that the

- *compound* ***accumulates*** *and* ***significant inhibition of neuropathy target esterase*** *or*
- *clinical/histopathological signs of delayed polyneuropathy occur at around the hen LD50 as determined in the single dose test.*

C i r c u m s t a n c e s i n w h i c h r e q u i r e d

These studies shall be performed for active substances of similar or related structures to those capable of inducing delayed polyneuropathy such as ***organophosphorus compounds.***

Neurotoxicity testing of pesticides

Acute

- Test No. 424: Neurotoxicity Study in Rodents
- Test No. 418: Delayed Neurotoxicity of Organophosphorus Substances Following Acute Exposure

Chronic

- Test No. 424: Neurotoxicity Study in Rodents
- Test No. 419: Delayed Neurotoxicity of Organophosphorus Substances: 28-day Repeated Dose Study

Developmental

- Test No. 426: Developmental Neurotoxicity Study

Problem formulation I: Challenges in testing

Animals	• Health issues
Dosing	• Too low • Bad spacing
Endpoints	• No measured • No reported
Statistics	• Inadequate statistical analysis
Interpretation	• No thorough and detailed • Lack of combination of observations

Challenges in testing: AN Example

Safety of Safety Evaluation of Pesticides: developmental neurotoxicity of chlorpyrifos and chlorpyrifos-methyl
Axel Mie, Christina Rudén, Philippe Grandjean
Environ Health. 2018; 17: 77, doi: 10.1186/s12940-018-0421-y

Re-evaluation of an industry-funded toxicity study that concludes that no selective effects on neurodevelopment occur even at high exposures.
Evidence from independent studies points to adverse effects of current exposures on cognitive development in children.
The analysis shown:

- Treatment-related changes in a **brain dimension measure** for chlorpyrifos **at all dose levels tested**, although **not been reported in the original test summary**.
- Dosage regimen that resulted in too low exposure of the nursing pups to detect any neurobehavioral effects
- The difference between raw data and conclusions in the test reports indicates a potential existence of bias that would require regulatory attention and possible resolution.

Problem formulation II: Application on mixtures

Combined exposure to multiple chemicals can lead to adverse effects on human health or the ecosystem, even if single substances in the mixtures ***are below their individual safety thresholds****.*

The assessment and management of chemical mixtures is only partly covered by current legislation, which focuses mostly on single substances.

In particular, while manufactured products such as pesticide formulations or cosmetic products are covered, unintentional mixtures which are coincidentally formed such as mixtures of contaminants e.g. in indoor air, are not consistently addressed.

Their composition is often unknown and changes over time, making them difficult to regulate. The assessment of unintentional mixtures is therefore usually limited to specific legislative sectors only, such as pesticide residues in food (EC website)

Problem formulation II: Application on mixtures

However, no studies on mixtures are available for regulatory use

Main challenges:

- The number of existing mixtures is tremendously big
- Consumers are exposed from all possible routes
- In many instances the accurate identification and quantitation of the chemicals in a mixture is impossible due to unstable composition and unknown chemicals (mainly in the environment)
- Difficulty in addressing interactions
- Further implication when consider non-chemical affecting parameters (e.g. radiations)

Problem formulation II: Application on mixtures

Reviewed Regulations and Directives of the EU law		Principals of the regulations	Mixture assessment for human health required?	Guidance for implementation of CRA available?
Biocidal products	Reg. EU No. 528/2012	Procedure: appr. of substance & author. of products	Yes	Yes ECHA, 2015a
CLP	Reg. EC No. 1272/2008	Classification of substances & mixtures	Yes	Yes ECHA, 2015b
Plant Protection Products	Reg. EC No. 1107/2009 Reg. EU No. 283/2013 Reg. EU No. 284/2013	Procedure: appr. of substance & author. of products	Yes	No
MRL`s	Reg. EC No. 396/2005	Setting of maximum residue levels	Yes	No
Medicinal Prod. for Human & Veterinary Use	Direct. 2001/83/EC Direct. 2001/82/EC	Author. procedure of products	Yes	Yes, drug interactions
Cosmetics	Reg. EC No. 1223/2009	Author. procedure of products	Yes	No
REACH	Reg. EC No. 1907/2006	Registration, authorisation of chemicals	No	No
Food and Feed Additives	Reg. EC No. 1333/2008 Reg. EC No. 1831/2003	Author. procedure of products	No	No

From EUROMIX Workshop 2019

Problem formulation II: Application on mixtures

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5.8.2. Supplementary studies on the active substance
Supplementary studies shall be carried out where they are necessary to further clarify observed effects taking into account the results of the available toxicological and metabolism studies and the most important exposure routes.
Such studies may include:

….
(f) studies on mixture effects.

Problem formulation II: Application on mixtures

- The EU regulation on maximum residue levels (MRLs) in food stipulates that **decisions on MRLs should take into account cumulative effects of pesticides** when the methods to assess such effects become available. In addition, the regulation covering the placing of pesticides on the market stipulates that pesticides should have no harmful effects – including cumulative effects – on humans.
- MRL Regulation requires developing new methodologies for CRA. Method development is the first necessary step to implement clear legal mandates and establish guidelines for a sound risk assessment.
- The **CAGs methodology** rests on the assumption that pesticides causing the same specific effects can produce joint, cumulative toxicity even if they do not have similar modes of action.
- EFSA: The full risk assessments of the cumulative effects of pesticides on the human **nervous** and thyroid systems are now **expected in June 2019**.

The RLRS concept

Two main elements:

- Mixtures of chemicals and possible other non-chemical stimuli
- Low doses – ADI levels

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Toxicology Letters

Volume 309, July 2019, Pages 33-34

Editorial

Toxicology for real-life risk simulation – Editorial preface to this special issue

Aristidis Tsatsakis, Marina Goumenou, Jyrki Liesivuori, Wolfgang Dekant, Antonio F. Hernández

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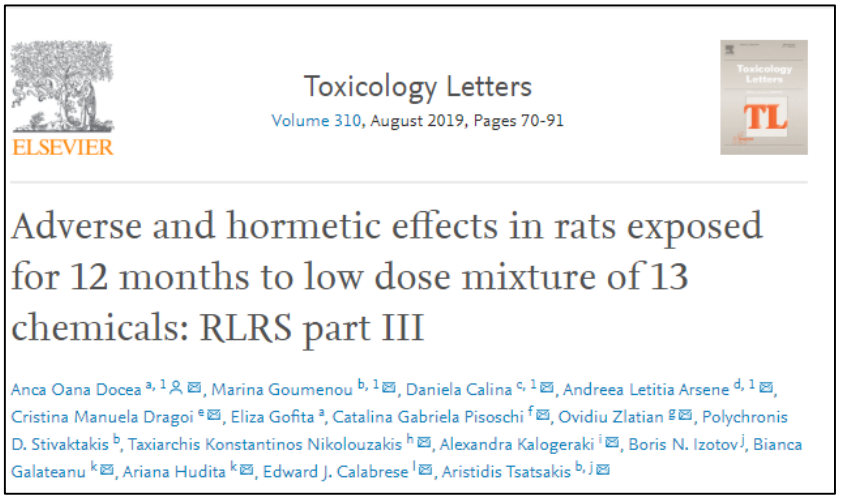

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Toxicology Letters

Volume 310, August 2019, Pages 70-91

Adverse and hormetic effects in rats exposed for 12 months to low dose mixture of 13 chemicals: RLRS part III

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Questions and ideas?