Pesticide Regulation in India

‘3R best practices’ for toxicological evaluation of pesticides

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About HSI

✧ Expert team: toxicology, ecotoxicology, pharmacology, biochemistry, neuroscience, endocrinology, law, regulatory science, etc.

✧ Present in India, China, Japan, Korea, Europe, United States, Canada, Mexico, Brazil and Latin America, Australia and beyond

✧ Working with research institutes, companies, government regulators and other stakeholders

HSI is the leading international NGO working to advance non-animal safety testing and bioscience research worldwide.
HSI science team representation

- India’s Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA)
- Core Committee of Indian Central Insecticide Board & Registration Committee
- PCD 19, Cosmetics committee at Bureau of Indian Standards
- OECD Test Guidelines, Chemicals & AOP development programs
- EU Competent Authorities for REACH and Classification and Labelling
- European Chemicals Agency Member State Committee, Endocrine Disruptors Expert Group, etc.
- USEPA Pesticide Program Workgroup on 21st Century Toxicology
- USTR Trade and Environment Policy Committee & TTIP negotiations
- Chinese Environmental Mutagen Society 21st Century Toxicology Group
- NTP Scientific Advisory Committee on Alternative Toxicological Methods
- European Union Reference Laboratory for Alternatives (EURL-ECVAM)
- International Cooperation on Cosmetics Regulation & national laws
- International Conference on Harmonization (via ICAPP)
- ... and others
Concept of “3R best practices”

✧ Strategies to Replace, Reduce or Refine animal use in toxicology
HSI’s vision

- Near-term reduction in animal use through uptake of “3R best practices” in product sector regulations (pesticides, etc.)

- Shift to a fully human biology-based paradigm based on understanding of “adverse outcome pathways” (AOPs)
Indian pesticide market overview

- Largest producer of pesticides in Asia
- 4th largest producer of crop protection chemicals in the world after USA, Japan & China
- Expected to grow at CAGR of 12% to reach $ 7.5 by 2019
- Insecticides make approximately 75% of the pesticide market followed by fungicides, herbicides & biopesticides
- According to the Pesticide Monitoring Unit of the Government of India; the crop protection market (as of FY12) comprises of:
  - 125 technical grade manufacturers, including 10 multinational companies
  - >800 formulators
  - 145,000 distributors
Pesticide regulation in India

Insecticides Act, 1968 & Rules, 1972

Manufacture, import, sale, transport, labelling & distribution

Central Insecticides Board (CIB)  Registration Committee (RC)
CIB

- Advises the Central Govt. on technical matter arising out of Insecticides Act & Rules
- Advises on manufacture, classification, residue limit, shelf life of insecticides
- Meets once every 6 months

RC

- Registers pesticides after verifying claims made by manufacturer/importer
- Specifies data requirement for safety testing & for packaging, transport, import, labelling
- Meets once every month
Current scenario of pesticides in India

- Chapter 3 (e) defines insecticides as:
  - Any substance in the schedule; or
  - Such other substances (including fungicides and weedicides as the Central Government may, after consultation with the Board, by notification in the official gazette, include in the schedule from time to time
- In practice, pesticides include: insecticides, weedicides, herbicides, fungicides, bio-pesticides and others
- 870 insecticides currently in schedule
- 272 pesticides currently registered in India and >2782 applications in process
Pesticide registration data requirements

- India an observer of OECD Council Decision regarding “Mutual Acceptance of Data” since March 2011
- New Indian Guidance Document effective from January 2015
  - Replaces Gaitonde Committee report from 1977
- Some reduction/refinement measures incorporated
- No contemporary OECD replacement/in vitro guideline tests adopted, leaving much room for improvement
3R best practices in regulation: European case study

- Between 2010-13, EU authorities, industry and HSI cooperated in the revision of registration data requirements for pesticides and biocides, achieving substantial (40+%) reductions in animal test requirements.

- Key achievements:
  - Removal of redundant *in vivo* study requirements, e.g., multiple exposure routes (acute dermal) and/or species (dog chronic).
  - Uptake of all applicable OECD 3R guideline methods and other scientifically supported alternative testing (and non-testing) strategies.
  - Endpoint-combining (e.g., *in vivo* micronucleus as part of a subchronic study).
  - Adopting more efficient study designs (e.g., extended 1-gen repro study).
  - Ingredient-based classification of formulated products (calculation approach).
Chronic toxicity: dog as second species

- Traditionally both rat and dog long-term studies have been required for all pesticide and biocide active ingredients.
- Retrospective reviews have for many years questioned the ‘added value’ of the dog study.
Significance of the Dog as ‘Second Animal Species’ in Toxicity Testing for Establishing the Lowest ‘No-Toxic-Effect Level’

L. M. Appelman and V. J. Feron
TNO-CIVO Toxicology and Nutrition Institute, P.O. Box 360, 3700 AJ Zeist, The Netherlands

Key words: second animal species; no-toxic-effect level.

The toxicity data of 66 compounds adequately tested in the rat (as ‘prime animal species’) and the dog (as ‘second animal species’) were studied to find out how many of these compounds would be classified as a lower ‘no-toxic-effect level’ (NTEL) than that found in the rat from a qualitative and quantitative point of view. The data would contribute to a better insight into the desirability of toxicity studies on the basis of daily intakes and permissible exposure levels in man are generally based on data from the rat.

For 44% of the compounds the NTEL in the dog was lower than that in the rat. The NTELs were calculated on the basis of a fixed dose (FD) of 10 mg/kg bw/day. As the arbitrary factor of 10, the adjusted NTELs were lower than the NTELs tested in sub-chronic studies, and for 80% of the compounds tested in chronic toxicity studies. In order to substitute an adjusted NTEL for a study in dogs should be judged.

A Comparison of the Results of Studies on Pesticides from 12- or 24-Month Dog Studies with Dog Studies of Shorter Duration

Karl P. Baetcke, Whang Phang, and Vicki Dellarco
Health Effects Division, Office of Pesticide Programs,
U.S. Environmental Protection Agency, Washington, D.C. 20460

The use of dogs as second species in regulatory testing of pesticides

Horst Spielmann, Ulrich Gerbracht

Part I: subacute, subchronic and chronic studies in the dog

Critical Reviews in Toxicology, 2009, 1-15, Early Online

REVIEW ARTICLE

A 1-year toxicity study in dogs is no longer a scientifically justifiable core data requirement for the safety assessment of pesticides

Werner Kobel, Ivana Fegert, Richard Billington, Richard Lewis, Karin Bentley, Werner Bomann, Phil Botham, Bernhard Stahl, Bennard van Ravenzwaay, and Horst Spielmann
Dog chronic, cont’d

REGULATORY AMENDMENT

✧ Data requirement deleted from Indian data requirements in 2011
  ✧ Also deleted in USA (2007), EU (2012-13), Brazil (2015) and Canada (2016)
✧ However, dog use will not end until all markets make this change
  ✧ Japan and Korea remain outliers

<table>
<thead>
<tr>
<th>Data requirement</th>
<th>3R best practice</th>
<th>US</th>
<th>EU</th>
<th>IN</th>
<th>BR</th>
<th>CA</th>
<th>KR</th>
<th>JP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic - dog</td>
<td>Deletion of data requirement</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>?</td>
<td>?</td>
</tr>
</tbody>
</table>
Evolving approaches to testing and assessment

- Significant progress in development and validation of 3R testing tools (cell and computer models), as well as non-testing approaches (waivers, read-across)
- Deployed according to integrated testing strategies using weight-of-evidence
- Ongoing paradigm shift based on AOP knowledge
- Regular updates to regulatory data requirements needed across product sectors (cosmetics, chemicals, pesticides) to keep pace with scientific progress
OECD guideline tests with *replacement* potential

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Test methods</th>
<th>OECD guideline (year)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin sensitization</td>
<td>DPRA, KeratinoSens (ARE-nrf2 luciferase), h-CLAT</td>
<td>442C &amp; 442D (2015), 442E (2016)</td>
</tr>
<tr>
<td>Skin absorption</td>
<td>Human post-surgical skin; RHE models</td>
<td>428 (2004)</td>
</tr>
<tr>
<td>Phototoxicity</td>
<td>3T3 Neutral Red Uptake (NRU)</td>
<td>432 (2004)</td>
</tr>
<tr>
<td>Acute fish toxicity</td>
<td>Fish embryo test</td>
<td>236 (2013)</td>
</tr>
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</table>

* In vitro methods currently represent about 50% of the OECD TGP work plan
### Other HSI suggestions for updating Indian req’s

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>‘3R best practice’</th>
<th>EU</th>
<th>IN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reproductive toxicity</td>
<td>Extended 1-generation study (EOGRTS) instead of 2-gen (- 1,200 animals = 46% reduction)</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Genetic toxicity, micronucleus</td>
<td>Assess in combination with a repeated dose toxicity study rather than 2 separate studies (- 80 animals)</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Acute toxicity, dermal</td>
<td>Waive study requirement if substance is unclassified (non-toxic) via oral route (- 30 animals)</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Carcinogenicity</td>
<td>Waive mouse study (- 400 animals)</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Developmental toxicity</td>
<td>Waive rat study if no concerns from rabbit developmental or rat EOGRTS (- 1,300 animals)</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Acute toxicity, fish</td>
<td>Tiered ‘threshold’ strategy (70% reduction)</td>
<td>✓</td>
<td></td>
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</tbody>
</table>
| ... etc.                              | Ongoing discussion of other available alternatives                                 |    | ...

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**EU**

-✓: Suggested

**IN**
Acute toxicity: dermal route

- Traditionally required for all pesticide and biocide active substances as well as for each end-use product
- Several large retrospective reviews of classifications comparing oral vs skin LD$_{50}$ data for pesticide actives and industrial chemicals

- Oral route classifications found to be more severe than dermal for 98% of pesticides and 99.9% of chemicals (Seidle, Prieto & Bulgheroni, ALTEX 2011, 28, 95-102)
- “Dermal acute systemic toxicity data almost never drive regulatory classification & labeling decisions in the chemicals, agrochemicals & biocides sectors.”
Acute toxicity testing of chemicals—Opportunities to avoid redundant testing and use alternative approaches

Stuart Creton¹, Ian C. Dewhurst², Lesley K. Earl³, Sean C. Gehen⁴, Robert L. Guest⁵, Jon A. Hotchkiss⁶, Ian Indans⁷, Michael R. Woolhiser⁸, and Richard Billington⁹

Examining the Regulatory Value of Multi-route Mammalian Acute Systemic Toxicity Studies

Troy Seidle¹, Pilar Prieto² and Anna Buigheroni²

¹Humane Society International, Research & Toxicology Department, Brussels, Belgium; ²In-Vitro Methods Unit, European Centre for the Validation of Alternative Methods, Konstanz, Germany; ³In-Vitro Methods Unit, European Centre for the Validation of Alternative Methods, European Commission Joint Research Centre, Ispra, Italy

Summary

Regulatory information requirements for pesticides call for submission of tests on up to three different exposure routes (oral, dermal, inhalation) for both active ingredients and products. Similar multi-route testing is required in the European Union and other regions for chemicals. To determine the value of acute toxicity testing by more than one route, concordances among regulatory classifications were examined. Out of 1,674 active substances, 4% were more severe than those derived from dermal data and 4% were more severe than for the inhalation route for 83% of pesticides examined.

Keywords: dermal toxicity, intelligent testing, LD₅₀, redundancy, regulatory tox

1 Introduction

Acute toxicity refers to adverse effects occurring following a single exposure to a substance or following multiple exposures. Acute toxicity studies are conducted to determine the potential harm to humans after a single exposure to a substance via the oral, dermal, and inhalation routes. These studies are integral to regulatory submissions for pesticides and chemicals.

Can acute dermal systemic toxicity tests be replaced with oral tests? A comparison of route-specific systemic toxicity and hazard classifications under the Globally Harmonized System of Classification and Labelling of Chemicals (GHS)

Nigel P. Moore⁴, David J. Andrew⁵, Donald L. Bjerke⁶, Stuart Creton⁷, David Dreher⁸, Thomas Holmes⁹, Pilar Prieto⁵, Troy Seidle⁵, Tim G. Rowan¹⁰

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⁴National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs), London, UK
⁵Center for Laboratory Animals, Hanover, UK
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⁷The European Union Reference Laboratory for Alternatives to Animal Testing (EURL-ELAMS), European Union-Joint Research Centre (JRC), Ispra, Italy
⁸European Union Reference Laboratory for Alternatives to Animal Testing (EURL-ELAMS), Food Safety and Consumer Protection, European Commission Joint Research Centre, Ispra, Italy
⁹European Partnership for Alternative Approaches to Animal Testing, Brussels, Belgium

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Abstract

Acute systemic toxicity tests (oral, dermal, inhalation) may not be required under the GHS. The present study investigated the reliability of acute dermal toxicity data as a substitute for oral data by comparing the acute oral and dermal toxicities for a large number of chemicals. The results indicate that acute dermal toxicities are often significantly lower than oral toxicities, suggesting that dermal toxicity data may be insufficient for replacing oral toxicity data in regulatory classifications.
Acute toxicity: skin

3R BEST PRACTICE

✧ Expand waiver criteria (OECD, 2016)
  ✧ For corrosives and gases
  ✧ For substances with oral LD50 (predicted \textit{in vitro}) >2000 mg/kg
  ✧ For substances with <10\% dermal penetration \textit{(in vitro)}

EU REGULATORY AMENDMENT

\textbf{Circumstances in which required}

The acute dermal toxicity of the active substance shall be reported unless waiving is scientifically justified (for example where oral LD$_{50}$ is greater than 2000 mg/kg). Both local and systemic effects shall be investigated.
Carcinogenicity: mouse as second species

- Traditionally both rat and mouse cancer bioassays have been required for all pesticide and biocide active ingredients.
- Retrospective reviews have for many years questioned the ‘added value’ of the mouse study.
Mouse carcinogenicity, cont’d

- Negligible impact of mouse bioassay data on ADI or cancer classification among 195 pesticide evaluations (Billington et al. Crit Rev Toxicol. 2010; 40, 35-49)

- Mouse ADIs for 10/195 (5.1%); lower mouse NOAEL resulted mainly from dose spacing vs. species sensitivity

- 3/195 classifications (1.5%) based on mouse-only data; all high-dose effects (up to 1 million times human exposure) and 2/3 considered ‘not likely human carcinogens’ by US EPA
Mouse carcinogenicity, cont’d

“The value of several individual tests that currently form part of the standard data package for plant protection products – in particular, the 1-year dog study and the mouse carcinogenicity study – is questionable. The PPR Panel suggests that the need for these studies should be reviewed.” (European Food Safety Authority, The EFSA Journal 2007; 449, 1-60)

“The conventional mouse carcinogenicity study does not add significant information for carcinogenicity evaluation over and above the use of the 24-month carcinogenicity assay in the male and female rat.” — Doe et al. Crit Rev Toxicol. 2006; 36, 37-68

Review of 273 chemicals: “Reasons are presented for favoring the rat as the species to be used.” — von Wittenau & Estes, Fundam Appl Toxicol. 1983; 3, 631-9

“The rat bioassay should be recognized as sufficient for confirmation of carcinogenic potential.” — Alden et al. Toxicol Pathol. 1996; 24, 722-5
Circumstances in which required

The long-term toxicity and carcinogenicity of all active substances shall be determined. If in exceptional circumstances it is claimed that such testing is unnecessary, that claim shall be fully justified.

Test conditions

A long-term oral toxicity study and a long-term carcinogenicity study (two years) of the active substance shall be conducted using rat as test species; where possible these studies shall be combined.

A second carcinogenicity study of the active substance shall be conducted using mouse as test species, unless it can be scientifically justified that this is not necessary. In such cases, scientifically validated alternative carcinogenicity models may be used instead of a second carcinogenicity study.
Toxicological studies for formulated products

- Calculations based on GHS additivity formula provide a protective, non-testing alternative to redundant acute systemic testing in vivo.

- Analysis of additivity formula predictions vs. 6-pack classifications of LD$_{50}$/LC$_{50}$ for 200 agrochemical formulations (Corvaro et al. 2016) found:
  - Oral LD$_{50}$ prediction: >86% accuracy
  - Dermal LD$_{50}$ prediction: >92-99% accuracy (no under-predictions)
  - Inhalation LC$_{50}$ prediction: >96% accuracy
Toxicological studies for formulated products

3R BEST PRACTICE

- Replace *in vivo* testing of formulated products with acute toxicity estimates using GHS additivity formula for acute oral, dermal and inhalation toxicity classifications
- Specify use of *in vitro* methods as the default for skin and eye corrosion/irritation, skin sensitization, and mutagenicity

EU REGULATORY AMENDMENT
## Status of HSI/industry negotiations with CIB-RC

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>‘3R best practice’</th>
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</thead>
<tbody>
<tr>
<td>Acceptance of validated/OECD alternatives</td>
<td>Appropriate language to be included in Indian Guidance Document to indicate the data acceptance from validated alternative methods</td>
</tr>
<tr>
<td>Skin &amp; eye irritation/corrosion</td>
<td>Reference to the validated OECD alternative test methods</td>
</tr>
<tr>
<td>Skin sensitization</td>
<td>Acceptance of standalone LLNA study data for registration purposes</td>
</tr>
<tr>
<td>Skin absorption</td>
<td>OECD TG 428 for consideration of the <em>in-vitro</em> penetration study as tiered approach for dermal toxicity studies</td>
</tr>
<tr>
<td>Inhalation toxicity</td>
<td>OECD TG 403 will be replaced with OECD TG 436 for Inhalation test</td>
</tr>
<tr>
<td>Reproductive toxicity</td>
<td>Preference for extended-1 generation study will be indicated in the guidance document</td>
</tr>
<tr>
<td>Avian acute toxicity</td>
<td>Agreed for one species instead of 2 species</td>
</tr>
<tr>
<td>Avian dietary toxicity</td>
<td>Waiver for formulation</td>
</tr>
<tr>
<td>Avian reproduction toxicity</td>
<td>Reduced from 2 species to 1 and possible waiver if avian acute LD50 &lt; 2000 mg/kg</td>
</tr>
<tr>
<td>Fish acute toxicity</td>
<td>Waiver for marine fish species requirement</td>
</tr>
</tbody>
</table>
### Topics of ongoing discussion...

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<tr>
<td>Reproductive toxicity</td>
<td>Removal of two generation studies and make Extended 1-generation study (EOGRTS) mandatory (- 1,200 animals = 46% reduction)</td>
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<td>Waive study requirement if substance is unclassified (non-toxic) via oral route (- 30 animals)</td>
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<td>Tiered ‘threshold’ strategy (70% reduction)</td>
</tr>
<tr>
<td>Waiver &amp; Bridging guidelines</td>
<td>Consistent to OECD recommend for waiving &amp; Bridging study guidelines from CIBRC</td>
</tr>
<tr>
<td>Tiered approach and mandatory</td>
<td>Pursue more strategic and scientific options in bringing in mandatory consideration of validated alternative methods and tiered approach for all eye &amp; skin studies</td>
</tr>
<tr>
<td>consideration of validated</td>
<td></td>
</tr>
<tr>
<td>alternative methods</td>
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</table>
Thank you for your attention!

Alokparna Sengupta
Deputy Director

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