Upholding Last Resort Principle for Chronic Fish Toxicity Under EU REACH - A Cosmetic Ingredient Case Study

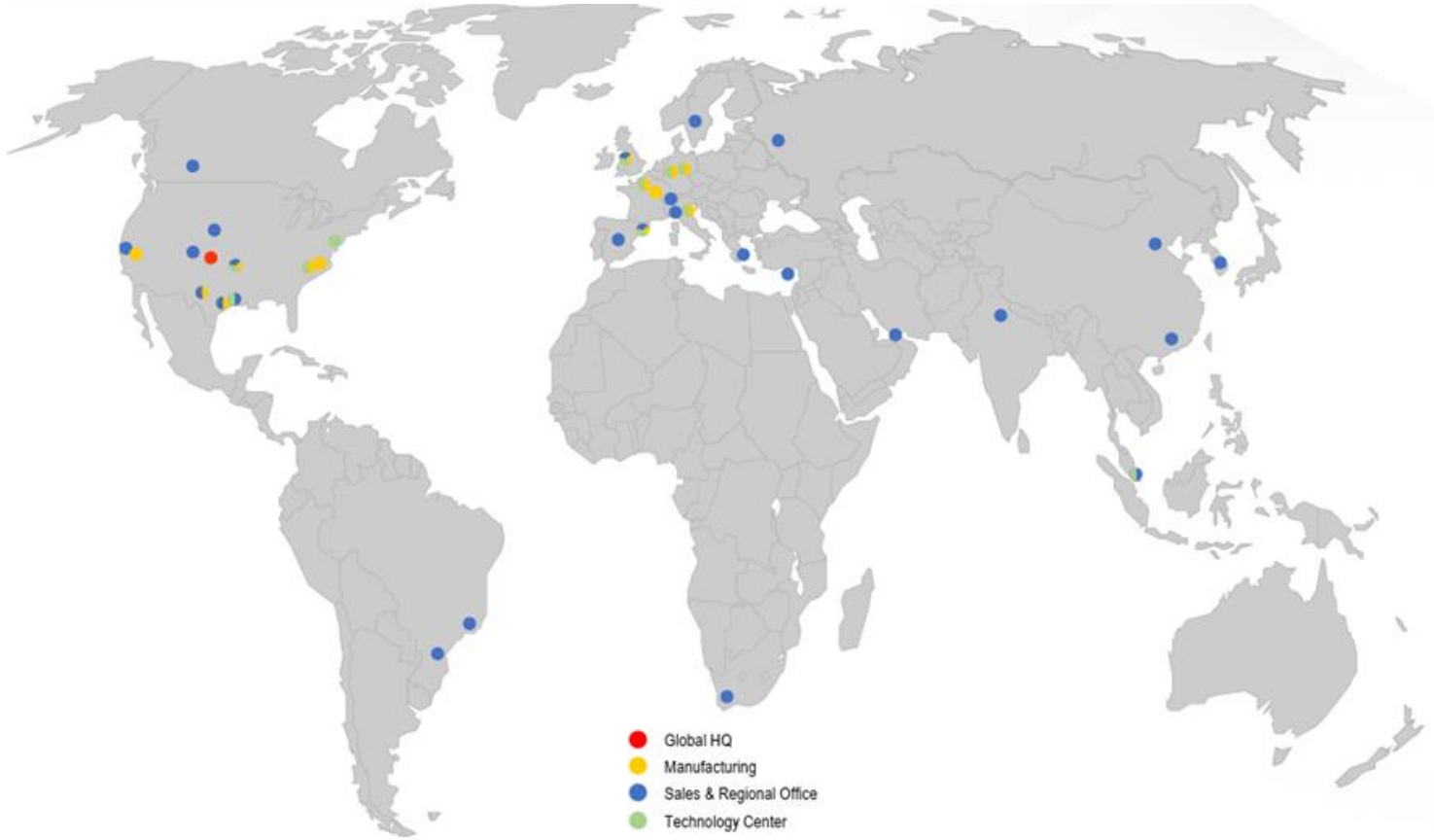
Jay Dawick
Senior Toxicology and Risk Assessment Manager
28 March 2024

AFSA COLLABORATION innospec

Outline

- Innospec Company Profile
- Case Study: C12-15 Alkyl Benzoate
    - Ingredient History & Use
    - EU REACH dossier compliance check (CCH)
    - Chronic Fish Toxicity (FELS Test)
        - Background
        - CCH Challenge
        - Innospec Strategy and Consortium Proposal
        - Outcome
    - Concluding Thoughts
- Q&A

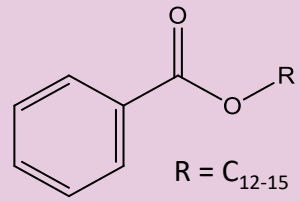
Company Profile Innospec Inc. (NASDAQ: IOSP)



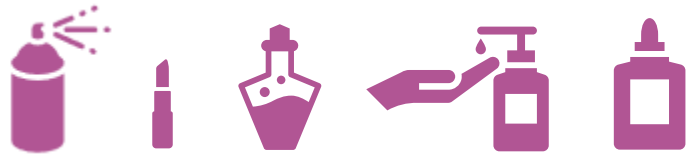
Financial Performance	Global Company	Core Business Units	Technology
$2 billion sales	2100 employees 23 Countries	Performance Chemicals Fuel Specialties Oilfield Services	Surface science Cross business exchange R&D Driven

C12-15 Alkyl Benzoate Use and History

- Invented by Innospec
- Reaction product of C12-15 alcohols and benzoic acid
- Non-volatile hydrophobic liquid UVCB
- Exclusive use cosmetic ingredient
- Used in APDO, lotions and moisturisers and sunscreens
- Long-standing safely profile and history of safe use
- Extensive global use
- EU REACH registered in 2010 Annex X (M/I>1000 tpa)



Benzoic acid, C12-15-alkyl esters
INCI: C12-15 Alkyl Benzoate
CAS: 68411-27-8
EC: 270-112-4



Substance property	Value
Appearance/state	Clear Liquid
Molecular weight	290 – 332 g/mol
Boiling Point	374°C
Melting point	-16.2 °C
Vapour pressure	<<0.1 Pa
Log Kow	8.0-9.6
Water solubility	≤ 2.47 µg/L

EU REACH Dossier Compliance Check (CCH)

►► **7 years:** ECHA issue dossier CCH draft decision

- **Multiple endpoints judged non-compliant**
  - Data not conforming to latest OECD TG methods
  - Read-across (RAx) rejected
  - Higher tier (eco)tox waivers rejected
- **Several new (eco)tox studies requested**

Link to CCH Decision

ECHA
EUROPEAN CHEMICALS AGENCY

9. **Growth inhibition study aquatic plants (Annex VII, Section 9.1.2.; test method: Alga, growth inhibition test, EU C.3./OECD TG 201) with the registered substance;**

10. **Short-term toxicity testing on fish (Annex VIII, Section 9.1.3.; test method: Fish, acute toxicity test, OECD TG 203) with the registered substance;**

11. **Long-term toxicity testing on aquatic invertebrates (Annex IX, Section 9.1.5.; test method: Daphnia magna reproduction test, EU C.20./OECD TG 211) with the registered substance;**

12. **Long-term toxicity testing on fish (Annex IX, Section 9.1.6.1.; test method: Fish, early-life stage (FELS) toxicity test, OECD TG 210) with the registered substance.**

You may adapt the testing requested above according to the specific rules outlined in Annexes VI to X and/or according to the general rules contained in Annex XI to the REACH Regulation. To ensure compliance with the respective information requirement, any such adaptation will need to have a scientific justification, referring and conforming to the appropriate rules in the respective annex, and adequate and reliable documentation.

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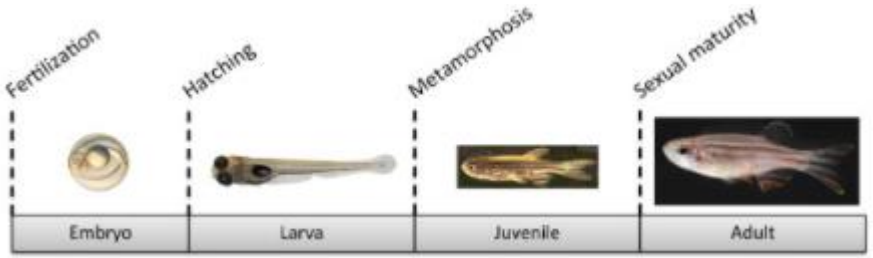
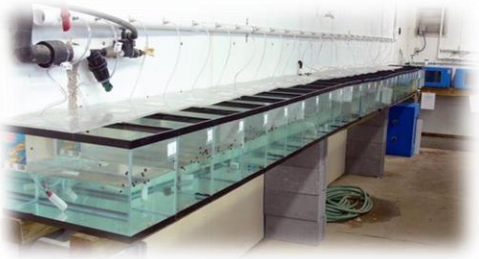
Chronic Fish Toxicity (FELS)



- **OECD 210 FELS Introduced >30 years ago as alternative to FFLC**
  - Primary test for estimating chronic (long-term) toxicity to fish
  - Used to support ERA and global chemical management
  - EU REACH SIR for substances M/I >100 tpa (Annex IX)

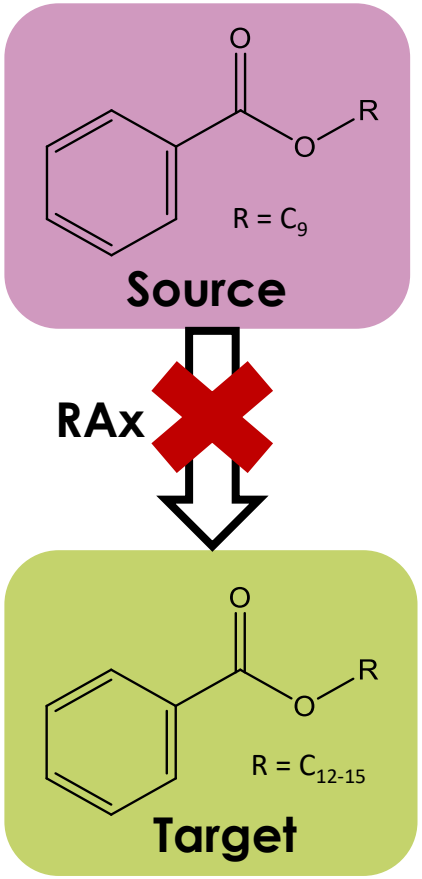


- **Involves testing on protected life stages of vertebrate animals**
- **Test design is labour, resource and animal intensive**
  - Study duration (in-life) 1-3 months depending on species
  - Requires at least 360 fish, but can be >700
  - Typical CRO costs are €70-150k depending on chemical/test design

- **Focus on apical endpoints and gross morphology i.e. survival, hatching, length**

Challenge

- **RAx Rejection**
  - Information missing to show source exhibits similar phys-chem and aquatic tox to target
  - Initial analogue chronically fish below limit of solubility (LoS) but not to daphnia
- **Both target and source not acutely toxic at limit of solubility**
  - Removed option to use species sensitivity or acute/chronic ratio (ACR)
- **Long-term aquatic toxicity testing critical for poorly water-soluble substances**
  - Require longer time to be taken up by test organisms and to reach steady state
- **However, ECHA open to read-across to cover <u>one</u> chronic endpoint:**

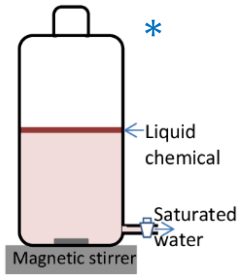


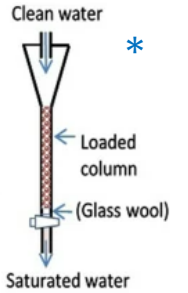
the aquatic toxicity potential of the registered target substance. ECHA notes also that in general the aquatic ITS cannot be applied for substances with low water solubility as further elaborated in section 11. below. However, ECHA acknowledges that as aquatic chronic data on the target substance becomes available, you may consider whether it can be used to *"support the read-across hypothesis and thus one chronic (long-term) ecotoxicological test can be avoided"*. The timeline given in this decision does allow for sequential testing of the aquatic endpoints if you wish to follow that approach. ECHA notes that all data supporting

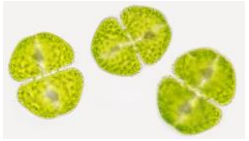
Innospec Strategy & Consortium Proposal

<u>Proposal to avoid chronic fish testing by:</u>

- **Executing new high quality non-vertebrate experimental testing on target:**
  - Water solubility (OECD 105; slow-stir/column elution)
  - Algal growth inhibition (OECD 201)
  - Long-term invertebrate (OECD 210)







- **Identifying and collating aquatic toxicity for sub-group of benzoate esters with different chain lengths**
  - Demonstrate and establish trends and potential break points in structure activity relationship (SAR)
- **Searching for new potential read-across analogues and use computational QSAR modelling**
  - New analogue data identified → not chronically toxic at LoS to fish or invertebrates
- **Review relevant literature on bioavailability "cut-off" limits for chronic toxicity of hydrophobic chemicals**

* Figures extracted from Birch et al 2019. Analytica chimica acta 1086 (2019): 16-28

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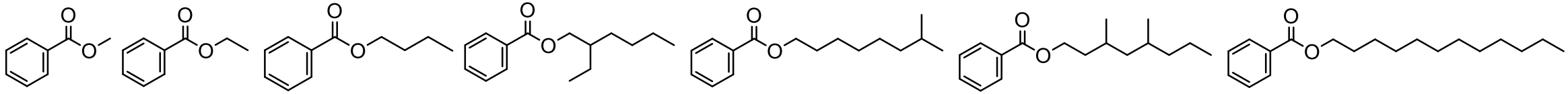
Innospec Strategy



Increasing hydrophobicity and decreasing water solubility

Endpoint	Ester 1	Ester 2	Ester 3	Ester 4	Ester 5	Ester 6	Ester 7
Water Solubility (µg/L)	2.1x10^6	7.2x10^5	30,000	400	≤78	≤69	≤2.5
Log Kow (KOWWIN v1.68)	1.8	2.3	3.3	5.2	5.7	5.6-6.6	7.2-8.7
Acute Aquatic Toxicity (µg/L)							
Acute Fish LC50	23,000	6700	1500	>660*	>1230*	>6500*	>1230* (RA Ester 5)
Acute Daphnia EC50	28,500**	27,100	4000	>125*	>2200*	>14*	>77*
Algae EC50	111,900	24,100	2900	>35*	>1000*	>50*	Test Proposed
Chronic Aquatic Toxicity (µg/L)							
Chronic Fish NOEC	**1788****	**919****	**237****	**16****	**42.8**	**≥47***	**RA Proposed**
Chronic Invert NOEC	34882**	16376**	3521**	173**	≥78*	≥39*	Test Proposed
Algae NOEC	62,400	8080	1500	≥35*	≥1000*	≥50*	Test Proposed

Additional Esters to show "Trend"

Former RAx Analogue

New RAx Analogue

Innospec Substance

Notes

*Result interpreted as >Limit of Solubility (LoS) or maximum achievable conc in test media

***In silico* (QSAR) prediction (ECOSAR v1.11)

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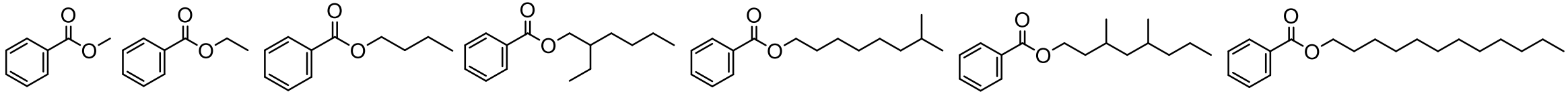
Innospec Strategy



Increasing hydrophobicity and decreasing water solubility

Endpoint	Ester 1	Ester 2	Ester 3	Ester 4	Ester 5	Ester 6	Ester 7
Water Solubility (µg/L)	$2.1x10^6$	$7.2x10^5$	30,000	400	≤78	≤69	≤2.5
Log Kow (KOWWIN v1.68)	1.8	2.3	3.3	5.2	5.7	5.6-6.6	7.2-8.7
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Algae EC50	111,900	24,100	2900	>35*	>1000*	>50*	>2.5*
Chronic Aquatic Toxicity (µg/L)							
Chronic Fish NOEC	**1788****	**919****	**237****	**16****	**42.8**	**≥47***	**≥47 (RA Ester 6)**
Chronic Invert NOEC	34882**	16376**	3521**	173**	≥78*	≥39*	>2.5*
Algae NOEC	62,400	8080	1500	≥35*	≥1000*	≥50*	>2.5*

Increasing acute toxicity, up to Ester 3 (cut-off)

Increase chronic (fish) toxicity, up to Ester 5 (cut-off)

New RAx (Ester 6)

New Data on Innospec Target Supporting RAx

Notes
*Result interpreted as >Limit of Solubility (LoS) or maximum achievable conc in test media
***In silico* (QSAR) prediction (ECOSAR v1.11)

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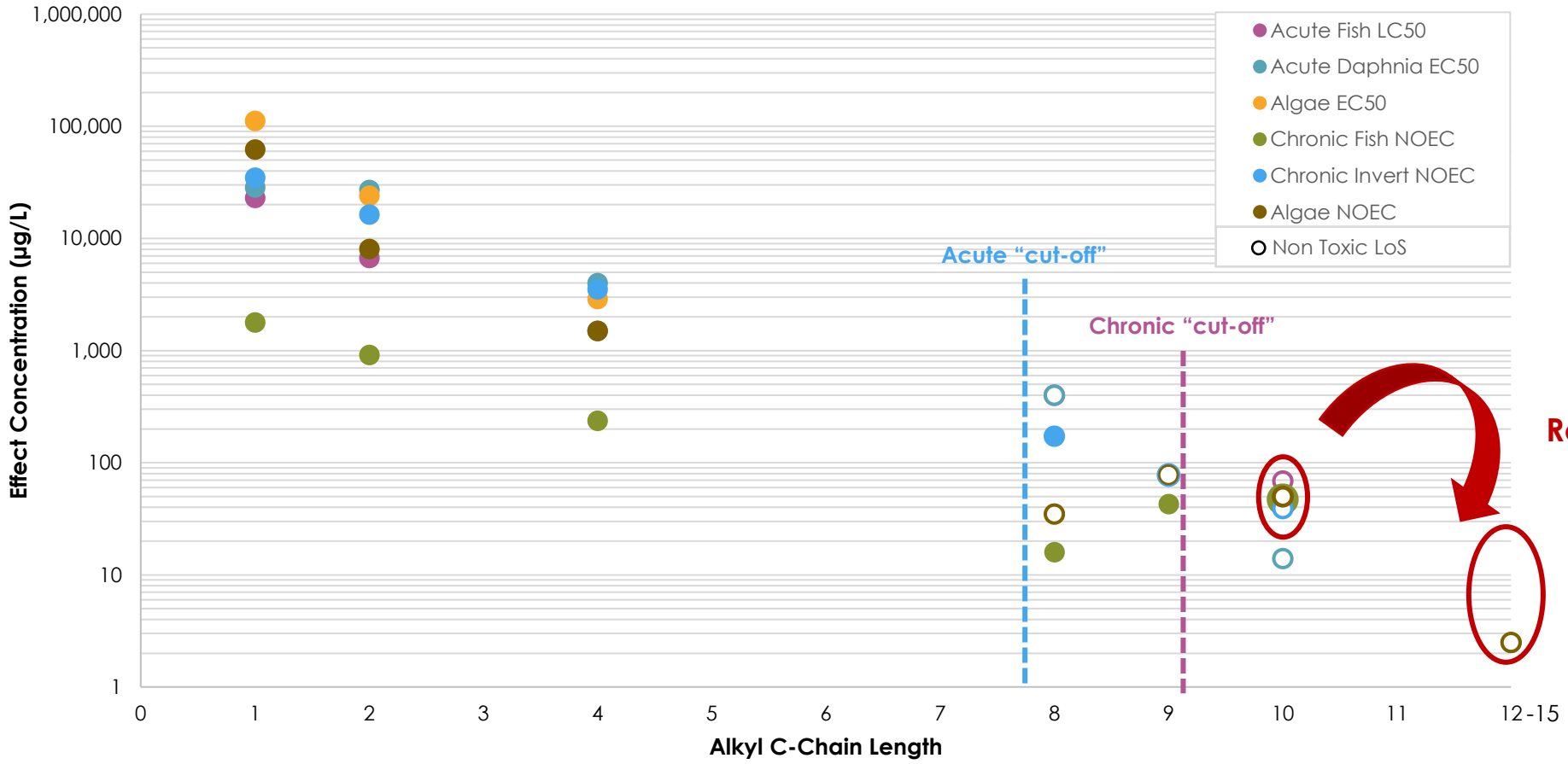
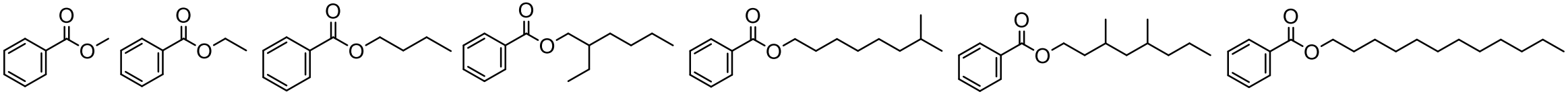
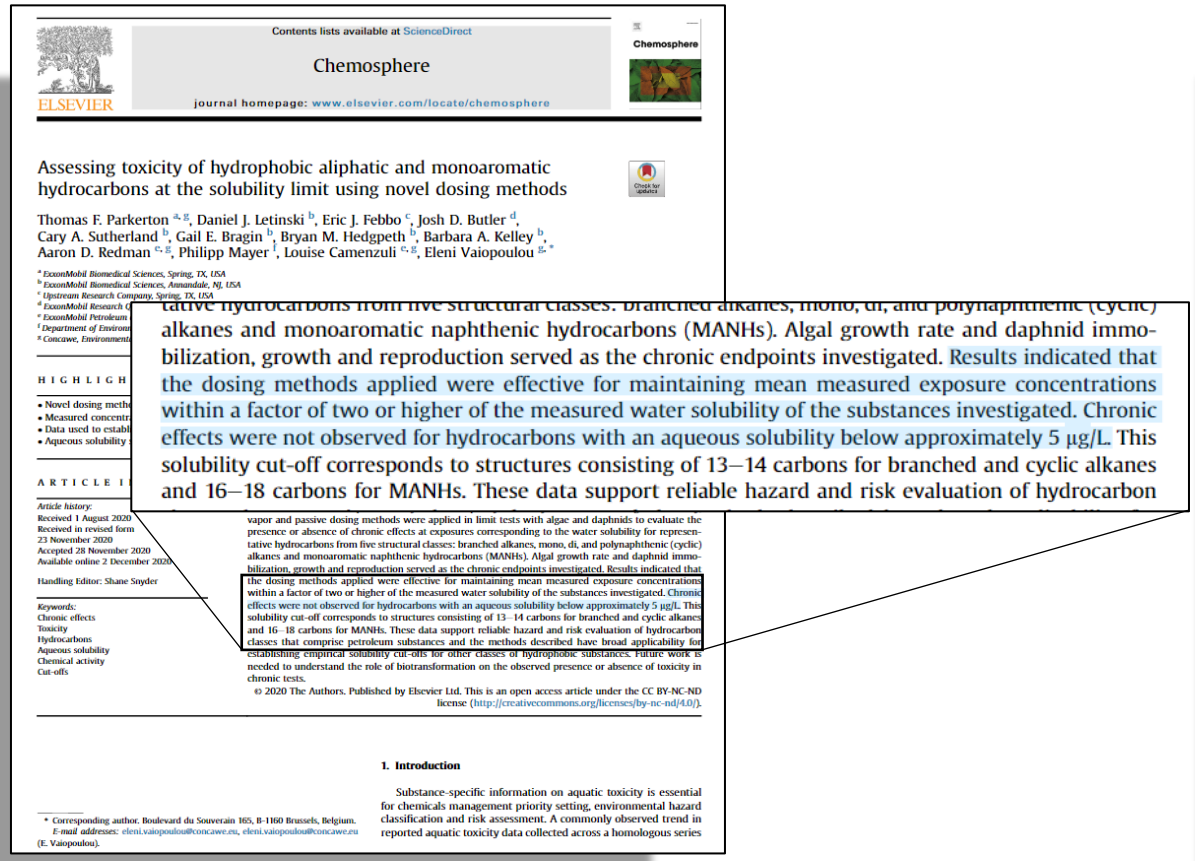
Innospec Strategy





Innospec Strategy

Example Literature Supporting Bioavailability "Cut-Off" for Chronic Toxicity of Very Hydrophobic Substances



Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Assessing toxicity of hydrophobic aliphatic and monoaromatic hydrocarbons at the solubility limit using novel dosing methods

Thomas F. Parkerton [a, g], Daniel J. Letinski [b], Eric J. Febbo [c], Josh D. Butler [d], Cary A. Sutherland [b], Gail E. Bragin [b], Bryan M. Hedgpeth [b], Barbara A. Kelley [b], Aaron D. Redman [c, g], Philipp Mayer [f], Louise Camenzuli [c, g], Eleni Vaiopoulou [g, *]

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[d] ExxonMobil Research Q
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[g] Concawe, Environment

HIGHLIGH

- Novel dosing meth
- Measured concentr
- Data used to establ
- Aqueous solubility

ARTICLE I

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Hydrocarbons
Aqueous solubility
Chemical activity
Cut-offs

tative hydrocarbons from five structural classes: branched alkanes, mono, di, and polynaphthenic (cyclic) alkanes and monoaromatic naphthenic hydrocarbons (MANHs). Algal growth rate and daphnid immobilization, growth and reproduction served as the chronic endpoints investigated. Results indicated that the dosing methods applied were effective for maintaining mean measured exposure concentrations within a factor of two or higher of the measured water solubility of the substances investigated. Chronic effects were not observed for hydrocarbons with an aqueous solubility below approximately 5 μg/L. This solubility cut-off corresponds to structures consisting of 13–14 carbons for branched and cyclic alkanes and 16–18 carbons for MANHs. These data support reliable hazard and risk evaluation of hydrocarbon

vapor and passive dosing methods were applied in limit tests with algae and daphnids to evaluate the presence or absence of chronic effects at exposures corresponding to the water solubility for representative hydrocarbons from five structural classes: branched alkanes, mono, di, and polynaphthenic (cyclic) alkanes and monoaromatic naphthenic hydrocarbons (MANHs). Algal growth rate and daphnid immobilization, growth and reproduction served as the chronic endpoints investigated. Results indicated that the dosing methods applied were effective for maintaining mean measured exposure concentrations within a factor of two or higher of the measured water solubility of the substances investigated. Chronic effects were not observed for hydrocarbons with an aqueous solubility below approximately 5 μg/L. This solubility cut-off corresponds to structures consisting of 13–14 carbons for branched and cyclic alkanes and 16–18 carbons for MANHs. These data support reliable hazard and risk evaluation of hydrocarbon classes that comprise petroleum substances and the methods described have broad applicability for establishing empirical solubility cut-offs for other classes of hydrophobic substances. Future work is needed to understand the role of biotransformation on the observed presence or absence of toxicity in chronic tests.

1. Introduction

Substance-specific information on aquatic toxicity is essential for chemicals management priority setting, environmental hazard classification and risk assessment. A commonly observed trend in reported aquatic toxicity data collected across a homologous series

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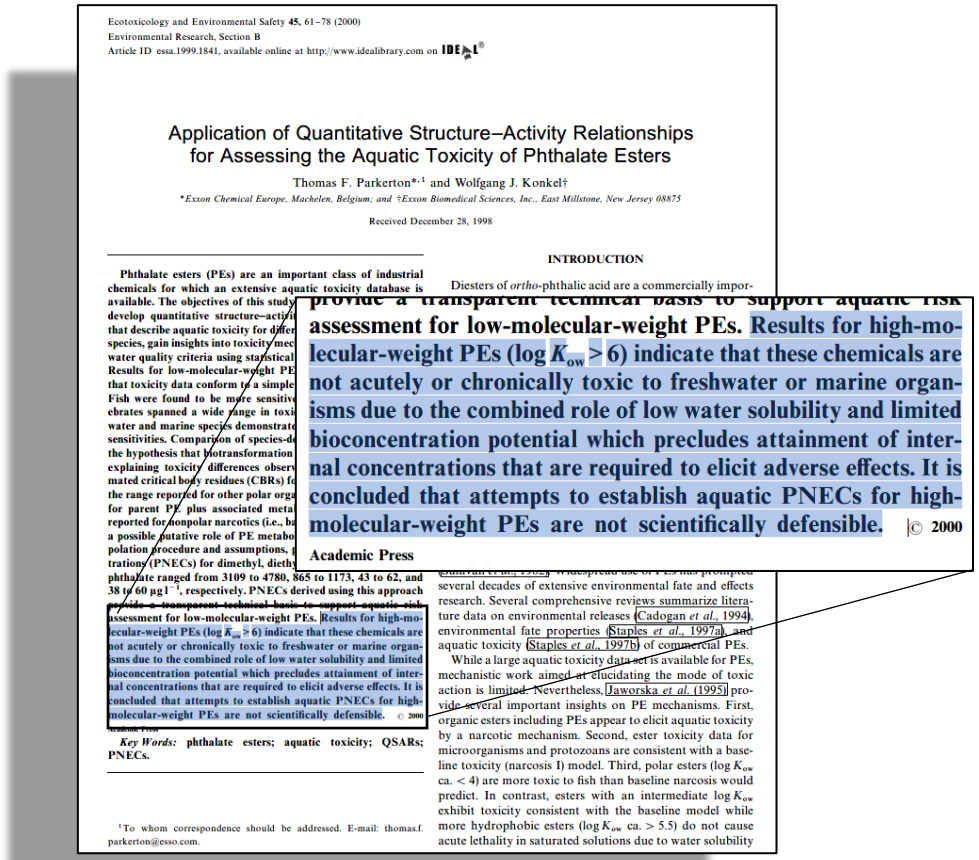


Ecotoxicology and Environmental Safety 45, 61–78 (2000)
Environmental Research, Section B
Article ID essa.1999.1841, available online at http://www.idealibrary.com on IDEAL®

Application of Quantitative Structure–Activity Relationships for Assessing the Aquatic Toxicity of Phthalate Esters

Thomas F. Parkerton*,[1] and Wolfgang J. Konkel†
*Exxon Chemical Europe, Machelen, Belgium; and †Exxon Biomedical Sciences, Inc., East Millstone, New Jersey 08875

Received December 28, 1998

Phthalate esters (PEs) are an important class of industrial chemicals for which an extensive aquatic toxicity database is available. The objectives of this study develop quantitative structure–activi that describe aquatic toxicity for differ species, gain insights into toxicity mec water quality criteria using statistical Results for low-molecular-weight PE that toxicity data conform to a simple Fish were found to be more sensitive ebrates spanned a wide range in toxi water and marine species demonstrate sensitivities. Comparison of species-d the hypothesis that biotransformation explaining toxicity differences obser mated critical body residues (CBRs) fo the range reported for other polar orga for parent PE plus associated meta reported for nonpolar narcotics (i.e., ba a possible putative role of PE metabo polation procedure and assumptions, trations (PNECs) for dimethyl, diethy phthalate ranged from 3109 to 4780, 865 to 1173, 43 to 62, and 38 to 60 μg l^{-1}, respectively. PNECs derived using this approach provide a transparent technical basis to support aquatic risk assessment for low-molecular-weight PEs. Results for high-molecular-weight PEs (log K_{ow} > 6) indicate that these chemicals are not acutely or chronically toxic to freshwater or marine organisms due to the combined role of low water solubility and limited bioconcentration potential which precludes attainment of internal concentrations that are required to elicit adverse effects. It is concluded that attempts to establish aquatic PNECs for high-molecular-weight PEs are not scientifically defensible. © 2000 Academic Press

Key Words: phthalate esters; aquatic toxicity; QSARs; PNECs.

[1]To whom correspondence should be addressed. E-mail: thomas.f.parkerton@esso.com.

INTRODUCTION

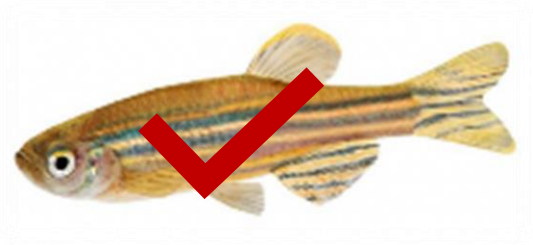
Diesters of *ortho*-phthalic acid are a commercially impor-

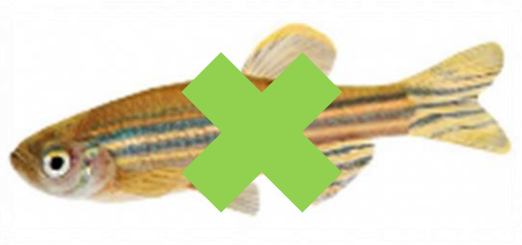
provide a transparent technical basis to support aquatic risk assessment for low-molecular-weight PEs. Results for high-molecular-weight PEs (log K_{ow} > 6) indicate that these chemicals are not acutely or chronically toxic to freshwater or marine organisms due to the combined role of low water solubility and limited bioconcentration potential which precludes attainment of internal concentrations that are required to elicit adverse effects. It is concluded that attempts to establish aquatic PNECs for high-molecular-weight PEs are not scientifically defensible. © 2000

several decades of extensive environmental fate and effects research. Several comprehensive reviews summarize literature data on environmental releases (Cadogan *et al.*, 1994), environmental fate properties (Staples *et al.*, 1997a), and aquatic toxicity (Staples *et al.*, 1997b) of commercial PEs.

While a large aquatic toxicity data set is available for PEs, mechanistic work aimed at elucidating the mode of toxic action is limited. Nevertheless, Jaworska *et al.* (1995) provide several important insights on PE mechanisms. First, organic esters including PEs appear to elicit aquatic toxicity by a narcotic mechanism. Second, ester toxicity data for microorganisms and protozoans are consistent with a baseline toxicity (narcosis I) model. Third, polar esters (log K_{ow} ca. < 4) are more toxic to fish than baseline narcosis would predict. In contrast, esters with an intermediate log K_{ow} exhibit toxicity consistent with the baseline model while more hydrophobic esters (log K_{ow} ca. > 5.5) do not cause acute lethality in saturated solutions due to water solubility

Outcome





Consortium

Disagreed with Innospec proposal (too risky)

Fear of further RAx rejection by ECHA?

Decided to commission new FELS test

innospec

Resigned from consortium and dropped LR role

Use new source substance to strengthen read-across

Developed Annex XI WoE and "opted-out" of new test

The Outcome

Other Registrants

COMMISSION FELS (LIMIT TEST)

NO ADVERSE EFFECTS AT LoS NOT CLASSIFIED

SUBSTANCE ANIMAL TESTED LAST RESORT NOT UPHELD

Early-2021

Mid-2021

The Outcome

Other Registrants

COMMISSION FELS (LIMIT TEST)

NO ADVERSE EFFECTS AT LoS NOT CLASSIFIED

SUBSTANCE ANIMAL TESTED LAST RESORT NOT UPHELD

Early-2021

Mid-2021

innospec

USE ENHANCED READ-ACROSS TO COVER FELS

NO ADVERSE EFFECTS BASED ON RA

"OPT-OUT" OF NEW CHRONIC FELS USING ANNEX XI WoE APPROACH

NOT ANIMAL TESTED LAST RESORT UPHELD

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The Outcome

Other Registrants

COMMISSION FELS (LIMIT TEST)

NO ADVERSE EFFECTS AT LoS NOT CLASSIFIED

SUBSTANCE ANIMAL TESTED LAST RESORT NOT UPHELD

Early-2021

Mid-2021

No Difference Env C&L or $PNEC_{aquatic}$

SAFE FOR ENV

innospec

USE ENHANCED READ-ACROSS TO COVER FELS

NO ADVERSE EFFECTS BASED ON RA

"OPT-OUT" OF NEW CHRONIC FELS USING ANNEX XI WoE APPROACH

NOT ANIMAL TESTED LAST RESORT UPHELD

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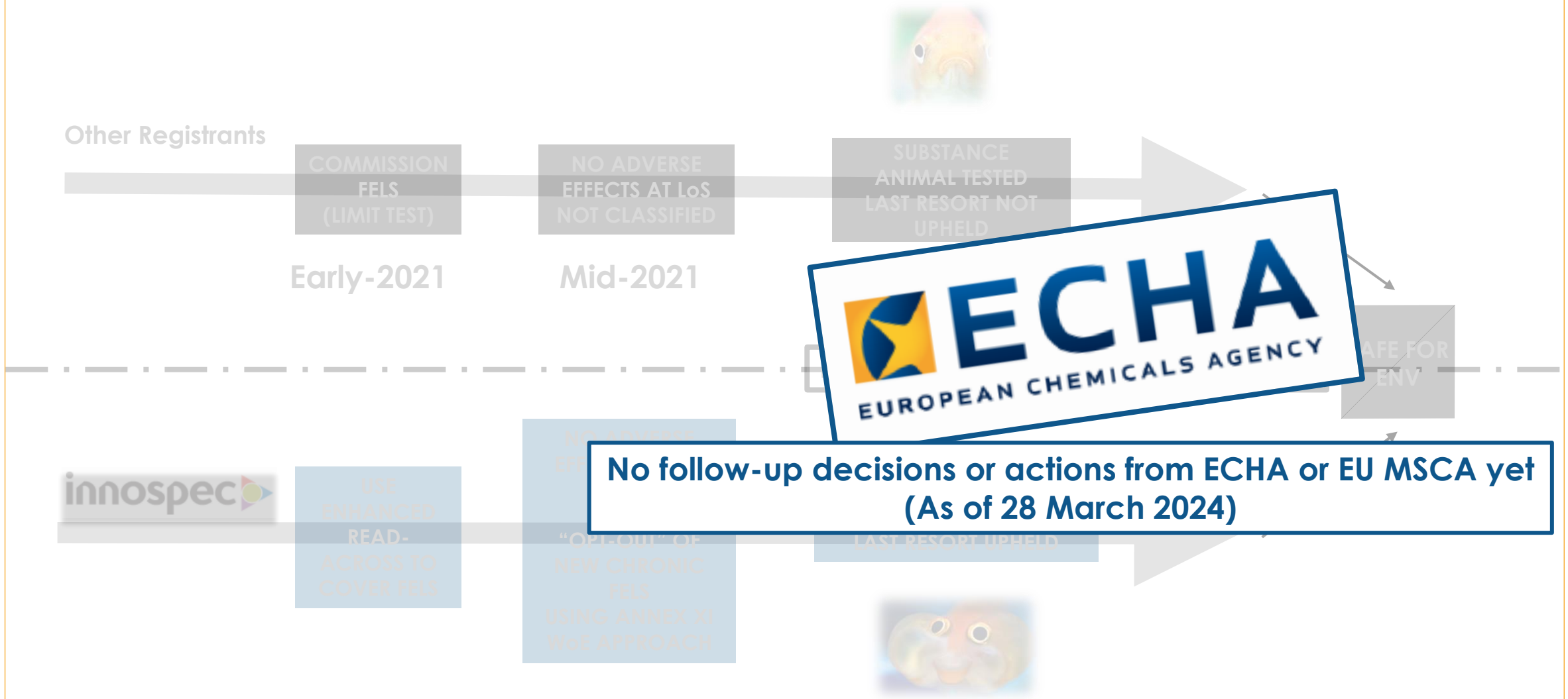
The Outcome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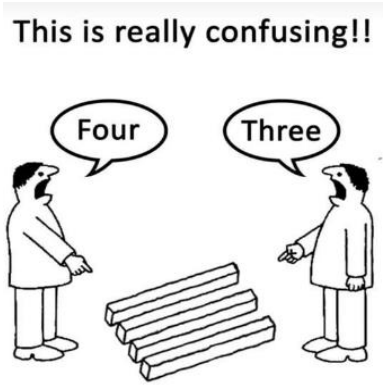
Early-2021
Mid-2021
ECHA
EUROPEAN CHEMICALS AGENCY
innospec
USE ENHANCED READ-ACROSS TO COVER FELS
"OPT-OUT" OF NEW CHRONIC FELS USING ANNEX XI WoE APPROACH
LAST RESORT UPHELD
AFE FOR ENV
No follow-up decisions or actions from ECHA or EU MSCA yet
(As of 28 March 2024)

Concluding Thoughts

- **Legal, commercial and ethical reasons to adhere to "last resort" resort principle**
  - Both the regulated and the regulator have clear obligations!
- **Read-across is powerful tool but robust scientific justification required**
  - High scientific bar for acceptance (fail once – reluctance to try again?)
  - Industry needs to:
    - Appreciate there may be different points of view/positions
    - Exploit all tools and methods available at our disposal
    - Do more and push hard(er) for acceptance
- **Case study demonstrates:**
  - Importance of exhausting all options before executing vertebrate animal testing
  - Possible to "opt-out" of new animal testing under EU REACH in case of disputes
  - Developing robust RAx often requires more time/effort (and potentially cost!) than testing



THANK YOU
QUESTIONS?

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The last resort requirement under REACH: From principle to practice

Donna S. Macmillan [a,*], Anders Bergqvist [b], Eleanor Burgess-Allen [c], Ian Callan [d], James Dawick [d], Benjamin Carrick [e], Graham Ellis [f], Roberto Ferro [g], Katy Goyak [h], Chantal Smulders [i], Ricky A. Stackhouse [j], Espe Troyano [k], Carl Westmoreland [l], Blanca Serrano Ramón [m], Vanessa Rocha [n], Xiaoling Zhang [k]

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ABSTRACT

REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) is a European Union regulation that aims to protect human health and the environment from the risks posed by chemicals. Article 25 clearly states that: "*[i]n order to avoid animal testing, testing on vertebrate animals for the purposes of this Regulation shall be undertaken only as a last resort.*" In practice, however, the standard information requirements under REACH are still primarily filled using animal studies.

This paper presents examples illustrating that animal testing is not always undertaken only as a last resort. Six over-arching issues have been identified which contribute to this: (1) non-acceptance of existing animal or non-animal data, (2) non-acceptance of read-across, (3) inflexible administrative processes, (4) redundancy of testing, (5) testing despite animal welfare concerns and (6) testing for cosmetic-only ingredients.

We, members of the Animal-Free Safety Assessment (AFSA) Collaboration, who work together to accelerate the global adoption of non-animal approaches for chemical safety assessment, herein propose several recommendations intended to aid the European Commission, the European Chemicals Agency and registrants to protect human health and the environment while avoiding unnecessary animal tests - truly upholding the last resort requirement in REACH.

1. Introduction

1.1. The Animal-Free Safety Assessment collaboration

The Animal-Free Safety Assessment (AFSA) Collaboration is a multi-stakeholder initiative developed to accelerate the global adoption of modern chemical safety assessment using non-animal approaches (AFSA, 2018). The AFSA Collaboration brings together leading industry and not-for-profit organisations with a shared goal to better protect people and our planet, by replacing animal testing with more predictive and relevant approaches. It has several key areas of activity, including:

- Increasing the understanding, uptake and acceptance of non-animal approaches in jurisdictions with or without cosmetic animal-testing bans through educational resources and targeted publications

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