



OCCUPATIONAL NEXT GENERATION RISK ASSESSMENT (NGRA) ON AN EXCLUSIVE USE COSMETIC INGREDIENT FOR EU REACH: A CASE STUDY ON C12-15 ALKYL BENZOATE

James Dawick

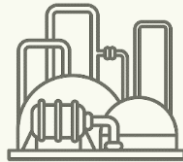
12th World Congress on Alternatives and Animal Use in the Life Sciences
Session 410 NAMs in Practice: Fit-for-Purpose NGRA Across Sectors

Animal Testing Conundrum: EU REACH and Cosmetics



EU CPR (EC) 1223/2009

Total **ban on animal testing** for **cosmetic products** and **ingredients** since 11 March 2013



EU REACH (EC) 1272/2008

Animal testing necessary to assess risks from **occupational exposure**, **non-cosmetic use(s)** and **environmental safety**

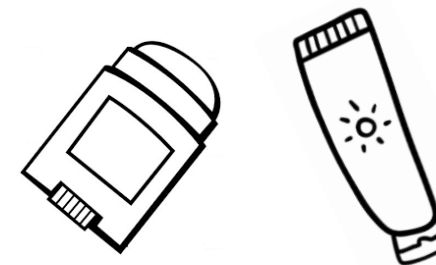
Article 25

Article 13

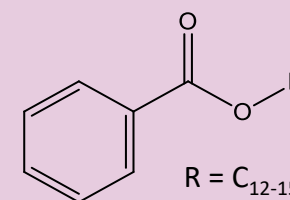
LAST RESORT!



C12-15 Alkyl Benzoate Use and History



- Invented by Innospec
- Reaction product of C12-15 alcohols and benzoic acid
- Non-volatile hydrophobic liquid UVCB
- Primary function: Emollient in skincare products
- Used extensively in APDO, lotions and moisturisers and as solubilizer/dispersant for sunscreen actives
- Long-standing safety profile in cosmetics for many years
- 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

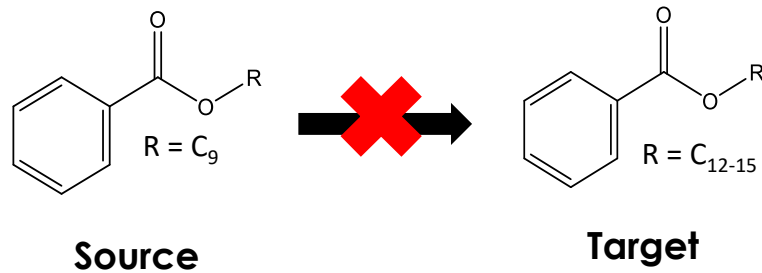
CAS: 68411-27-8

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

ECHA Compliance Check

- **Rejected read-across** approach
- Historical studies **not conforming to latest guidance/methods**
- Higher tier tox study **waivers rejected**
- Animal studies essential to **ensure worker protection**



- Innospec committed to **no further animal testing**
- Initial attempts to **strengthen read-across rejected**
- **Alternative Occupational NGRA strategy developed** and shared with co-registrants
 - Rejected and decided to proceed with animal testing
- **Innospec dropped lead-registrant role and resigned** from consortium
 -which enabled us to **“opt-out”** of animal testing according to Articles 11(3)c and 19(2)c of EU REACH

ECHA
EUROPEAN CHEMICALS AGENCY

CONFIDENTIAL 1 (36)

Helsinki, 9 November 2017

Addressee: Innospec Limited
Innospec Manufacturing Park
CH65 4EY, Ellesmere Port
United Kingdom
Decision number: CCH-D-2114375450-52-01/F
Substance name: BENZOIC ACID, C12-15-ALKYL ESTERS
EC number: 270-112-4
CAS number: 68411-27-8
Registration number: 01-2119825559-27-0001
Submission number: AV349087-16
Submission date: 19/10/2012
Registered tonnage band: Over 1000

DECISION ON A COMPLIANCE CHECK

Based on Article 41 of Regulation (EC) No 1907/2006 (the REACH Regulation), ECHA requests you to submit information on:

1. **Water solubility** (Annex VII, Section 7.7.; test method: EU A.6./OECD TG 105)
2. **In vitro gene mutation study in bacteria** (Annex VII, Section 8.4.1.; test method: Bacterial reverse mutation test, EU B.13/14. / OECD TG 471) with the registered substance;
3. **In vitro cytogenicity study in mammalian cells** (Annex VIII, Section 8.4.2., test method: OECD TG 473) or **in vitro micronucleus study** (Annex VIII, Section 8.4.2, test method: OECD TG 487) with the registered substance;
4. **In vitro gene mutation study in mammalian cells** (Annex VIII, Section 8.4.3.; test method: OECD TG 476 or TG 490) with the registered substance, provided that the studies requested under 2. and 3. have negative results;
5. **Sub-chronic toxicity study** (90-day), oral route (Annex IX, Section 8.6.2.; test method: EU B.26./OECD TG 408) in rats with the registered substance;
6. **Pre-natal developmental toxicity study** (Annex IX, Section 8.7.2.; test method: EU B.31./OECD TG 414) in a first species (rat or rabbit), oral route with the registered substance;
7. **Pre-natal developmental toxicity study** (Annex X, Section 8.7.2., column 2; test method: EU B.31./OECD TG 414) in a second species (rat or rabbit), oral route with the registered substance;
8. **Extended one-generation reproductive toxicity study** (Annex X, Section 8.7.3.; test method: EU B.56./OECD TG 443) in rats, oral route with the registered substance specified as follows:
 - Ten weeks pre-mating exposure duration for the parental (P0) generation;

Occupational NGRA – What is it?

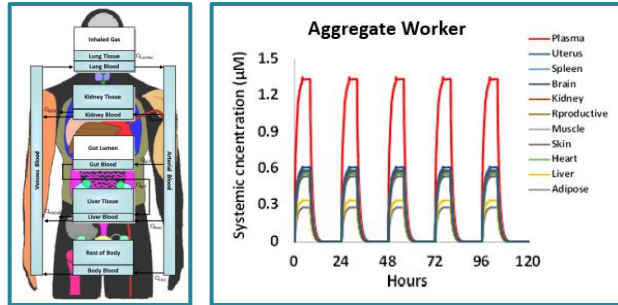


Occupational NGRA is exposure-led, hypothesis-driven approach integrating New Approach Methodologies (NAMs) to demonstrate worker safety without animal testing



Occupational NGRA Work Flow

Occupational Exposure C_{max} Estimation



Worker Activity Scenario

External Exposure Model

Dermal
Inhalation

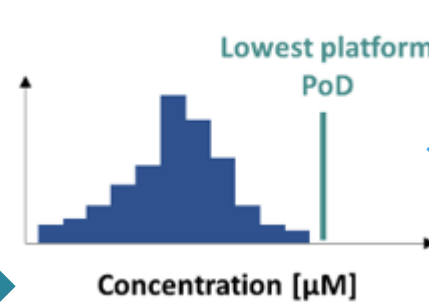
Internal Exposure (PBK Model)

In vitro liver clearance
In vitro PPB
In vitro skin pen/metabolism

C_{max}
Estimate

Bioactivity Exposure Ratio (BER)

Population C_{max} estimate



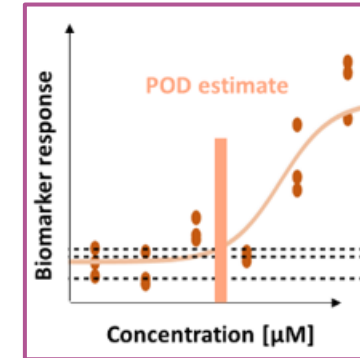
$$\text{BER} = \text{PoD} / C_{\text{max}}$$

$\text{BER} > 1$
Bioactivity Leading to Adverse Effects Can Be Ruled Out

$\text{BER} < 1$
Bioactivity Leading to Adverse Effects Cannot Be Ruled Out

Lowest PoD

In vitro Bioactivity PoD Platform



DART PoD

Reprotracker and DevToxQP

IPP Agonist/Antagonist PoD

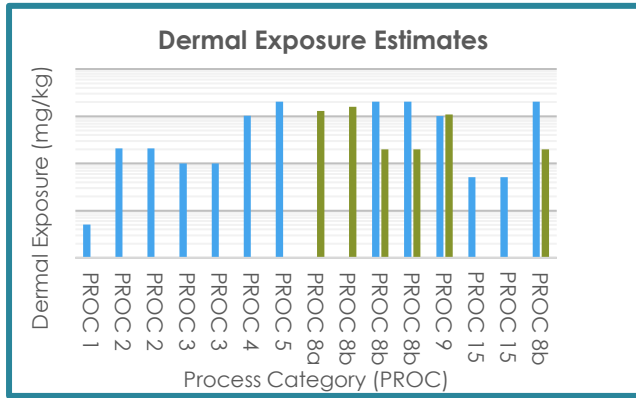
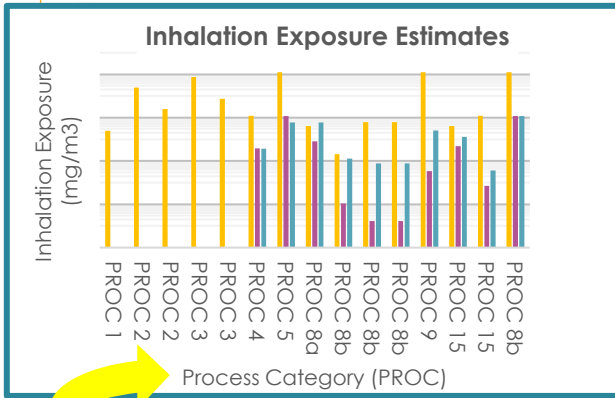
Cell Stress Panel PoD

HTTr PoD

Exposure Assessment



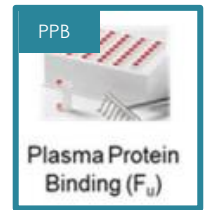
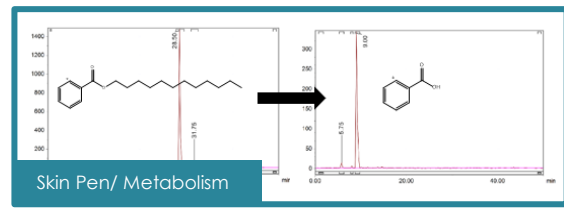
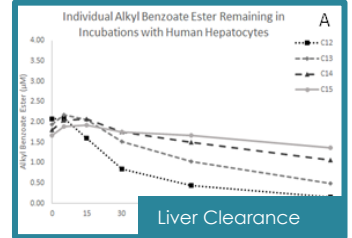
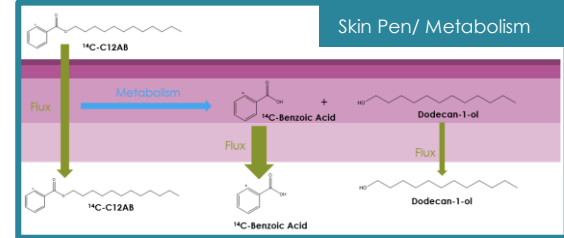
External Exposure Modelling



Worker Activity Scenario (Formulation)



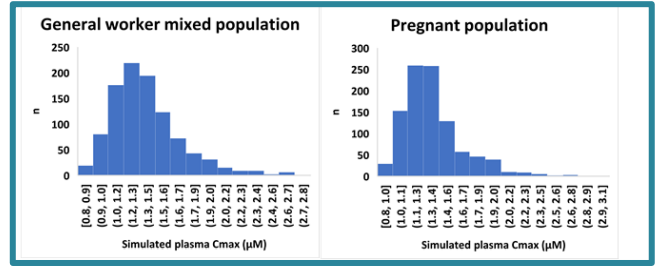
In vitro ADME for input to PBK



Internal Exposure (PBK Population Model)



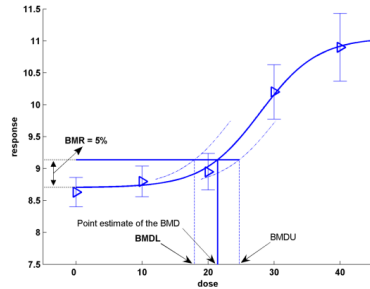
SimulationsPlus



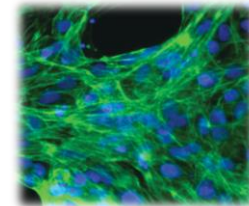
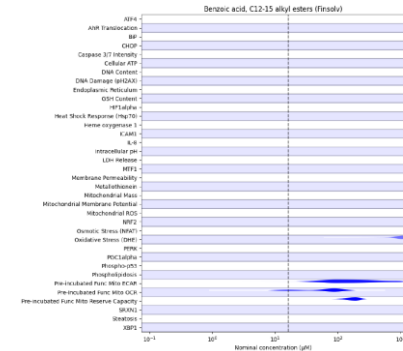
Population type (n=1000)	Median (µM)	5th %ile [µM]	Mean [µM]	95th %ile [µM]
General population	1.32	0.97	1.38	1.98
Pregnant population	1.29	0.98	1.34	1.90



Bioactivity Assessment



Bioactivity Platform	Cell Line/Type	Nominal PoD (µM)
Cell Stress Panel	Human HepG2	16 (6.4*)
IPP	Various	>10
HTTr (BIFROST)	Human MCF7	1400
HTTr (BIFROST)	Human HepG2	77
HTTr (BIFROST)	Human HepaRG	2200
HTTr (BMDEExpress)	Human MCF7	617
HTTr (BMDEExpress)	Human HepG2	155
HTTr (BMDEExpress)	Human HepaRG	>5000
Dev-ToxQP	Human iPSC	>30
Reprotracker	Human iPSC	>30



***Lowest Nominal PoD adjusted based on analytical dose confirmation**

- Large suite of *in vitro* bioactivity assays on **human cell lines** to derive PoD with good biological coverage
- Module further supplemented to include screening coverage for **DART endpoints**
- Lowest nominal PoD from CSP assay was 6.4 µM** adjusted following analytical determination of concentration in test media (accounts for poor solubility, plastic binding and instability)

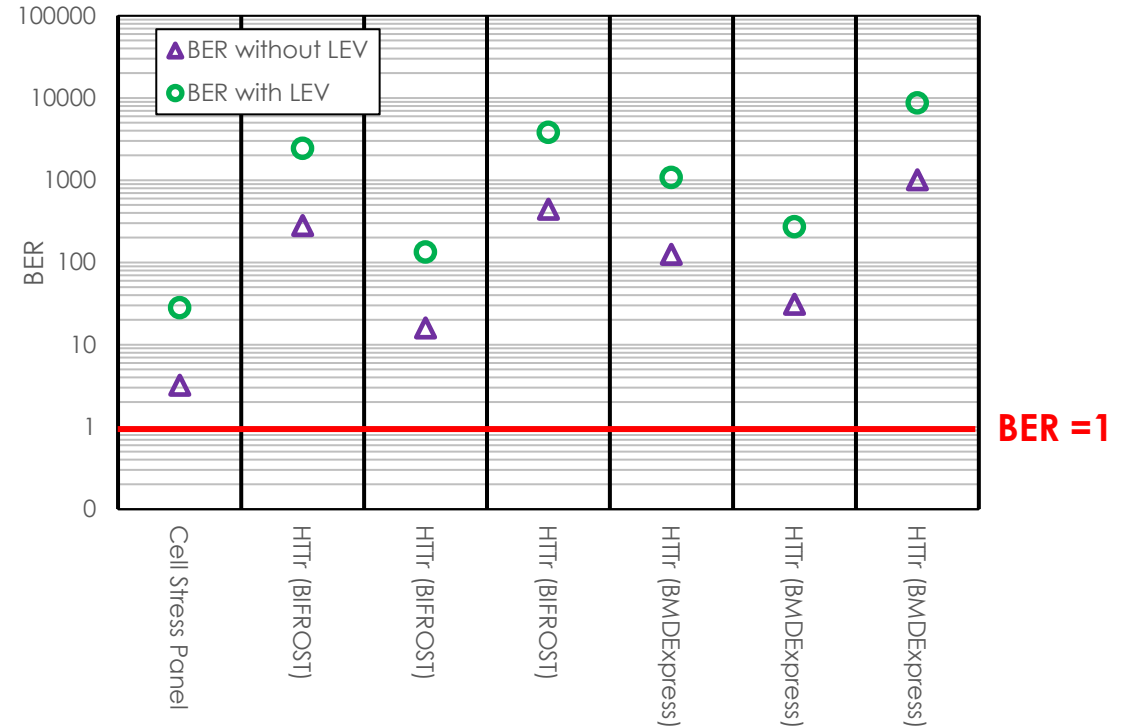
Risk Assessment



Bioactivity Platform	BER without LEV	BER with LEV
Cell Stress Panel	3.2	28
IPP	>5*	>43*
HTTr (BIFROST)	282	2435
HTTr (BIFROST)	16	134
HTTr (BIFROST)	444	3826
HTTr (BMDEExpress)	125	1073
HTTr (BMDEExpress)	31	270
HTTr (BMDEExpress)	1010	8696
Dev-ToxQP	>15*	>52*
Reprotracker	>15*	>52*

Lowest PoD

*included for comparative purposes as no PoD derived



Occupational BER >1 for All Activities

Includes Aggregate Exposure from Multiple Tasks During Typical 8h Shift
 Conservative Inhalation Exposure Predominant Driver of Potential Risk/Low BER's

Conclusion

- Occupational NGRA successfully executed in EU REACH context
 - BER >1 for all worker activities
 - Confidently assign a **low-risk conclusion** using **conservative human-relevant** approaches
 - Animal testing to prove worker safety not justified** from a scientific/ethical standpoint
 - Upheld last resort principle** as per Articles 13 and 25 EU REACH
- Innospec EU REACH dossier updated in May 2023
 - NAMs also used to **enhance/strengthen read-across**
 - No feedback** from ECHA yet
- EU REACH co-registrants are **actively animal testing**
 - Innospec have “opted-out”**
 - Parallel **animal vs non-animal** approach will be interesting future case study!
- Not stopping here.....additional work to **enhance NGRA** includes:
 - Occupational exposure monitoring** (especially for inhalation)
 - Compare **Occupational vs Consumer** exposure(s) and BER's

		Activity (NAM-based toxicodynamics)		
		High	Medium	Low
Potential Systemic Availability (NAM-based toxicokinetics, based on ADME properties)	High	H	H	L
	Medium	H	M	L
	Low	M	L	L

Berggren et al 2023 Reg Tox and Pharm, Vol 142,105431



vs



Acknowledgements



**Lauren Kavanagh
Ian Callan**



**Matt Dent
Steve Gutsell
Hequn Li
Ruth Pendleton
Hugh Barlow
Sophie Cable
Iris Muller
Jade Houghton
Joe Reynolds
Predrag Kukic
Gordon Riley
Richard Cubberley
Sue Martin**



**Peter Sladen
Mike Crookes
Oliver Warwick**



**Chris Waine
Dan Threlfall**

THANK YOU

QUESTIONS?