EUROTOX 2023
LJUBLJANA – SLOVENIA
10–13 SEPTEMBER 2023

ToxMinds
THINKING AHEAD IN TOXICOLOGY

THE USE OF METABOLOME ANALYSIS IN SUPPORTING THE CATEGORY JUSTIFICATION OF FATTY ACID ALKANOLAMIDES UNDER THE EU REACH REGULATION

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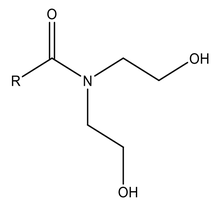
Introduction

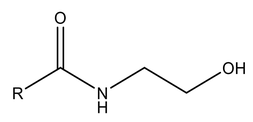
Fatty acid alkanolamides (FAA) are non-ionic surfactants predominantly used in cosmetics, household products and the lubricant sector. The following substances are covered in the FAA category:

DEA-derived FAA



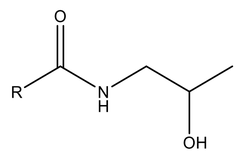
R = C11-unsatd. DEA (EC No. 262-114-9); Mono-constituent; 1-10 tpa
R = C12 DEA (EC No. 204-393-1); Mono-constituent; 100-1000 tpa
R = C8-18 and C18-unsatd. DEA (EC No. 931-329-6); UVCB; > 1000 tpa
R = C12-18 and C18-unsatd. DEA (EC No. 931-335-9); UVCB; > 1000 tpa
R = C16-18 and C18-unsatd. DEA (EC No. 271-653-9); UVCB; 100-1000 tpa
R = C18-unsatd. DEA (EC No. 700-972-2); UVCB; 100-1000 tpa

MEA-derived FAA



R = C8-18 and C18-unsatd. MEA (EC No. 931-330-1); UVCB; > 1000 tpa
R = C12-18 and C18-unsatd. MEA (EC No. 931-338-5); UVCB; > 1000 tpa
R = C16 MEA (EC No. 208-867-9); Mono-constituent; > 10-100 tpa
R = C16-18 MEA (EC No. 203-883-2); UVCB; 100-1000 tpa
R = C18-unsatd. (EC No. 947-890-5); UVCB; 10-100 tpa

MIPA-derived FAA



R = C8-10 MIPA (EC No. 915-384-3); UVCB; 10-100 tpa
R = C8-18 and C18-unsatd. MIPA (EC No. 931-596-9); UVCB; > 1000 tpa
R = IsoC18 MIPA (EC No. 431-540-9); mono-constituent; 100-1000 tpa

The FAA category includes monoethanolamine- (MEA), diethanolamine- (DEA) and monoisopropanolamine- (MIPA) derived fatty acid alkanolamides with varying alkyl chain lengths.

To meet the EU REACH registration information requirements, the FAA REACH consortium established a grouping approach with the hypothesis that MEA, DEA and MIPA derived FAA have common structures with comparable structure activity and metabolites as well as common toxicokinetic and toxicological properties. To strengthen the grouping justification, the FAA consortium conducted, as Tier 1 of a 2-tiered testing programme, a series of 14-day dose range finding and OECD TG 422 combined repeated dose toxicity studies with reproductive/developmental toxicity screening with substances of all FAA subcategories.

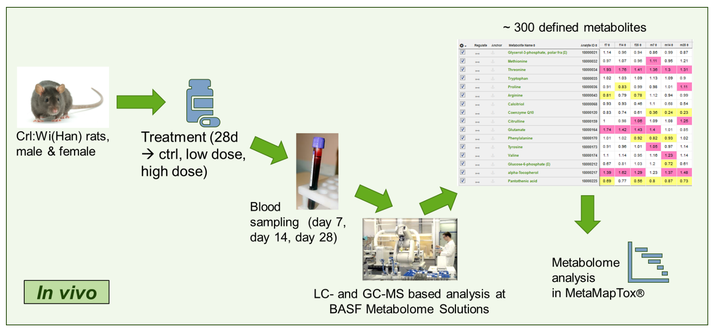
To enhance the quality and quantity of the generated *in vivo* data from a biological perspective, the FAA consortium contracted BASF Metabolome Solutions GmbH to conduct metabolomics analyses of plasma samples taken prior to necropsy of the 14-days dose range finding and the OECD TG 422 study in rats treated with representative MEA, DEA and MIPA derived FAA substances for 14 and 28 days. The metabolome investigated in this study included 404 endogenous metabolites in plasma covering a broad range such as carbohydrates, amino acids, fatty acids and hormones.

Metabolomics: Definition and Methodology

METABOLOMICS

- Study of metabolites (intermediates and products of metabolism usually defined as a molecule < 1.5 kDa) present in a biological sample
- Provides a direct functional readout of cellular activity and physiological status

METHODOLOGY AND BUILD-UP OF THE METAMAPTOX® DATABASE



1. Extraction from biological samples
2. Separation (GC, HPLC, SPE before chromatography in case of hormones and catecholamines)
3. Detection (Mass spectometry)
4. Identification and quantification
5. Metabolomics analysis
   - Metabolite profile
   - Pattern ranking
   - Treatment correlation

Study Outline

Table 1: Overview of Tier 1 FAA testing programme

	DEA-FAA					MEA-FAA				MIPA-FAA		
	C12 DEA	C8-18 and C18-unsatd. DEA	C12-18 and C18-unsatd. DEA	C16-18 and C18-unsatd. DEA	C18-unsatd. DEA	C8-18 and C18-unsatd. MEA	C12-18 and C18-unsatd. MEA	C16 MEA	C16-18 MEA	C8-10 MIPA	C8-18 and C18-unsatd. MIPA	IsoC18 MIPA
Annex	IX	X	X	IX	IX	X	X	VIII	IX	VIII	X	IX
OECD 421/422	(✓)	(✓)	(✓)	(✓) + MB	(✓)	(✓) +MB	(✓)	✓	(✓) + MB	(✓)	(✓) + MB	(✓)
Caco-2												
ToxProfiler												
ReproTracker												

- **Blood samples (14d DRF – 4M/4F per dose group (low- and high-dose); 28d OECD 422 - 5M/5F per dose group (low- and high-dose); all control samples (individual – no pooling)**
  - Amides, C16-18 and C18-unsatd., N,N-bis(hydroxyethyl) ('C16-18 and C18 DEA')
  - Amides, C8-18 and C18-unsatd., N-(hydroxyethyl) - ('C8-18 and C18 MEA')
  - Amides, C16-18 and C18-unsatd., N-(hydroxyethyl) - ('C16-18 and C18 MEA')
  - Amides, C8-18 and C18-unsatd., N-(2-hydroxypropyl) - ('C8-18 and C18 MIPA')

Metabolite profile assessment
Biological interpretation

BACKGROUND

- Analysis of specific metabolic changes for each substance in support of the biological interpretation.

OUTCOME

Table 2: Summary of metabolites changes and profile strengths of the test substances relative to the study controls

Abbreviated name	Females (LD)			Females (HD)			Males (LD)			Males (HD)		
	No.	%	Profile strenght	No.	%	Profile strenght	No.	%	Profile strenght	No.	%	Profile strenght
C16-18 & C18-unsatd. DEA (DRF)	33	**8,17**	1,09	64	**15,84**	1,54	16	3,96	0,9	52	**12,87**	1,49
C16-18 & C18-unsatd. DEA (OECD TG 422)	17	4,21	0,92	16	3,96	1,03	20	4,95	0,85	17	4,21	0,79
C8-18 and C18-unsatd. MEA (OECD TG 422)	-	-	-	-	-	-	7	1,73	0,71	29	**7,18**	0,98
C16-18 MEA (DRF)	4	0,99	0,75	4	0,9	0,75	15	3,71	0,89	29	**7,18**	1,10
C16-18 MEA (OECD TG 422)	20	4,95	0,82	27	**6,68**	0,97	8	1,98	0,72	8	1,98	0,9
C8-18 and C18-unstad. MIPA (DRF)	26	**6,44**	1,08	17	4,21	0,84	15	3,71	0,81	13	3,22	1,02
C8-18 and C18-unstad. MIPA (OECD TG 422)	11	2,72	0,8	11	2,72	0,9	8	1,98	0,75	31	**7,67**	1,02

Dark blue values indicate a metabolome change below the false positive rate / **Bold black values** indicate a metabolome change above the false positive rate at p<0.05

- The number of significant metabolome changes varied from no changes to minor changes (compared to control, p<0.05 based on 404 metabolites)
- Generally dose-dependent if changes above false positive rate

Pattern ranking analysis
Mode of Action identification

BACKGROUND

- A comparison of the metabolite changes of a test substance with all predefined final patterns in the MetaMapTox® database which are predictive for particular modes of action

OUTCOME

Table 3: Comparison against predefined toxicity patterns in the MetaMapTox® database

Abbreviated name	Females (LD)	Females (HD)	Males (LD)	Males (HD)
C16-18 & C18-unsatd. DEA (DRF)	EQUIVOCAL / MISMATCH	EQUIVOCAL / MISMATCH	EQUIVOCAL / MISMATCH	WEAK MATCH (liver – paracetamol like toxicity)
C16-18 & C18-unsatd. DEA (OECD TG 422)	EQUIVOCAL / MISMATCH	EQUIVOCAL / MISMATCH	EQUIVOCAL / MISMATCH	EQUIVOCAL / MISMATCH
C8-18 and C18-unsatd. MEA (OECD TG 422)	-	-	EQUIVOCAL / MISMATCH	MISMATCH
C16-18 MEA (DRF)	MISMATCH	EQUIVOCAL / MISMATCH	MISMATCH	MISMATCH
C16-18 MEA (OECD TG 422)	EQUIVOCAL / MISMATCH	WEAK MATCH (liver toxicity)	MISMATCH	MISMATCH
C8-18 and C18-unstad. MIPA (DRF)	EQUIVOCAL / MISMATCH	MISMATCH	MISMATCH	MISMATCH
C8-18 and C18-unstad. MIPA (OECD TG 422)	EQUIVOCAL / MISMATCH	EQUIVOCAL / MISMATCH	MISMATCH	MISMATCH

- For most substances no matches with patterns of toxicity were identified
- Only weak matches, equivocal findings and mostly mismatches
- In the case of 2 substances, weak matches for liver toxicity (sex-specific; not dose-dependent)

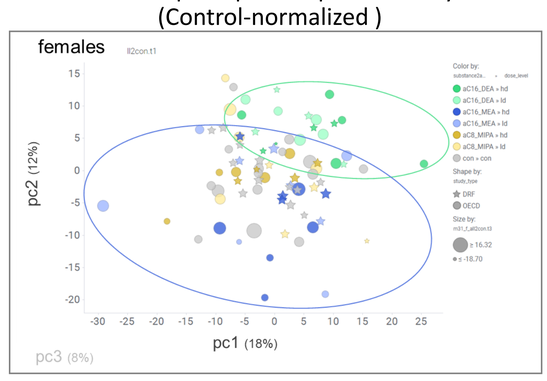
Treatment correlation analysis
Total profile comparison

BACKGROUND

- A comparison of the metabolome profile of a test substance with the metabolome profiles of all other substances in the MetaMapTox® database enabling a total profile comparison
- Testing for the identification of potential subclusters within the group of test substances

OUTCOME

Figure 1: Overview of principal component analysis in females (Control-normalized)



- Control and treated samples are not clearly separated indicating only few metabolites changes
- Small difference between treatments was observed (most significant with C16-18 and C18-unsatd. DEA)
- PCA analysis indicated no sub-clustering within the group of test substances

Conclusions

- No to only very weak metabolome changes (compared to control, p<0.05 based on 404 metabolites)
- Only a very slight metabolome change suggesting a liver effect detected for C16-18 and C18unsatd. DEA-FAA (females) and C8-18 and C18unsatd. MIPA-FAA (males) in high dose samples of DRF
  - Low dose treatment correlated with high dose treatment
  - Match *in vivo* findings for both substances (i.e., slight, non-adverse liver weight increases in both sexes)
- Principal component analysis and hierarchical clustering did not identify any clustering/sub-clustering of the substances
  - Slight separation of HD DRF C16-18 and C18unsatd. DEA-FAA and C8-18 and C18unsatd. MIPA-FAA indicating some degree of non-significant biological activity
- Identification of Mode of Action
  - No strong correlations with patterns of toxicity
- Absence of significant metabolome changes are in line with the *in vivo* Tier 1 (OECD 421/422) findings