The combined use of metabololome data from rat plasma and liver cell system for determination of toxicological equivalency of enantiomers

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Objective

To evaluate if metabololome data can be used to establish toxicological equivalency between racemates and optically active compounds.

Case study: Dimethenamid and its more active herbicidal enantiomer (Dimethenamid-P).

Background and Introduction

BASF’s Experimental Toxicology and Ecology unit and metanomics GmbH implemented a project to predict toxicological risks by measuring metabololome profiles in rat plasma from short term repeated dose studies in rats. Within this context the MetaMap®Tox database with more than 500 reference compounds was developed.

Metabololome analysis in plasma was performed after 7, 14, and 28 days and relative levels of endogenous low molecular weight compounds (metabolites) in treated rats versus controls were analyzed. Sets of common metabolite level changes were established to characterize several toxicological modes of action (so called MoA patterns).

Some chemicals can be produced as mixtures of optically active compounds or as pure enantiomers. It is known that chiral properties of molecules may have an influence on biological properties. To investigate if metabololomics may be used as a technology to establish toxicological equivalency we studied the metabolic effect of Dimethenamid (1:1 mixture of R:S enantiomers) and Dimethenamid-P. The latter is the more active herbicidal isomer of Dimethenamid, a herbicide belonging to the chemical class of chloroacetamides. The toxicity to mammalian species of both compounds has been studied and is essentially similar.

Methods

4-week studies with Crl:WI (Han) rats
- adapted OECD-407
- Blood sampling after 7, 14, and 28 days
- Sample preparation
- polar fraction
- LC-MS/MS, GC-MS
- Metabolite levels of the dosed rat samples related to the respective metabolite concentration of the control group
- Uploading in MetaMap®Tox database for data analysis

MoA patterns in MetaMap®Tox

Patterns were established based on at least three different chemicals sharing a common toxicological mode of action. Such patterns were checked by an expert panel and further validated against the data base.

Fit to patterns

Using an established algorithm, the similarity of the metabolite profile of the test compound with the pre-defined patterns in MetaMap®Tox (> 120 patterns) was determined and evaluated by an expert panel.

Dose groups

Dimethenamid and Dimethenamid-P were administered to the animals via diet. Dose levels were 1500 ppm (LD) and 4000 ppm (HD).

Results

Identification of target organs and MoAs

- No biologically relevant differences concerning the identification of target organs and MoAs were found.
- Strengths of profile for Dimethenamid and Dimethenamid-P are virtually identical.

Metabolite changes

- Dimethenamid-P caused slightly stronger metabolite changes in males and females than Dimethenamid.
- 75% of those regulated metabolites belong to the same lipids, fatty acids and related metabolite family.

Profile comparison with reference compounds

No quantitative or qualitative relevant differences were shown between Dimethenamid and Dimethenamid-P.

Conclusion

- No biologically relevant differences concerning the identification of target organs and MoAs were found between both compounds.
- Mode of Action pattern screening of Dimethenamid and Dimethenamid-P identified the liver as main target organ.
- Indirect effects on thyroid patterns are assessed to be due to an increased breakdown in the liver of thyroid hormones, based on liver enzyme induction.
- No detected differences in the qualitative toxicological assessment between both compounds were found.

<table>
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<th>Treatment</th>
<th>Strength</th>
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<th>rank</th>
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<td>1.33</td>
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</table>

- Using total profile comparison (>1000 different treatments), the best correlation for the metabolite profile of Dimethenamid HD in animals of both sexes was the more active isomer Dimethenamid-P HD.
- Strengths of profile for Dimethenamid and Dimethenamid-P are virtually identical.

No quantitative or qualitative relevant differences were shown between Dimethenamid and Dimethenamid-P.