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Metabolite profiles of rats in repeated dose toxicological studies after oral and inhalative exposure

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HIGHLIGHTS

- Comparison of metabolic profiles in rat plasma after repeated inhalative and oral exposure for 6 toxicants with known toxicological mode of action.
- Route of exposure dependent metabolic response for toxicants with weak metabolome changes (low profile strengths) e.g. female rats dosed inhalatively with Aniline or Tetrahydrofurane.
- Route of exposure independent metabolic response for toxicants with profound metabolome changes (high profile strengths), e.g. rats dosed with chloroform or 2-methoxyethanol.
- Link of inhalatively tested compounds to MetaMap®-Tox database with plasma-metabolome and toxicity data of rats for more than 550 reference compounds, dosed orally in an adapted OECD 407 protocol.

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ABSTRACT

The MetaMap®-Tox database contains plasma-metabolome and toxicity data of rats obtained from oral administration of 550 reference compounds following a standardized adapted OECD 407 protocol. Here, metabolic profiles for aniline (A), chloroform (CL), ethylbenzene (EB), 2-methoxyethanol (ME), *N*,*N*-dimethylformamide (DMF) and tetrahydrofurane (THF), dosed inhalatively for six hours/day, five days a week for 4 weeks were compared to oral dosing performed daily for 4 weeks. To investigate if the oral and inhalative metabolome would be comparable statistical analyses were performed. Best correlations for metabolome changes via both routes of exposure were observed for toxicants that induced profound metabolome changes. e.g. CL and ME. Liver and testes were correctly identified as target organs. In contrast, route of exposure dependent differences in metabolic profiles were noted for low profile strength e.g. female rats dosed inhalatively with A or THF. Taken together, the current investigations demonstrate that plasma metabolome changes are generally comparable for systemic effects after oral and inhalation exposure. Differences may result from kinetics and first pass effects. For compounds inducing only weak changes, the differences between both routes of exposure are visible in the metabolome.

Abbreviations: A, aniline; CL, chloroform; DMF, *N*,*N*-dimethylformamide; EB, ethylbenzene; LOAEL, lowest observed adverse effect level; PWC, pairwise comparison; ME, 2-methoxyethanol; MoA, mode of action; MTD, maximum-tolerated dose; THF, tetrahydrofurane.

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1. Introduction

Metabolite profiling in plasma (analysis of endogenous low molecular weight metabolites such as amino acids, carbohydrates, fatty acids, lipids and others) was developed in the last decades and can be applied within toxicology studies to elucidate changes of endogenous metabolites describing the physiological state of an organism, e.g. after administration of test substances (Lindon et al., 2004a; van Ravenzwaay et al., 2007). Two technologies are mostly applied: (i) metabolic profiling by NMR as described e.g. by Griffin and Bollard (2004) or Lindon et al. (2004b) and (ii) metabolic profiling by LC–MS and GC–MS as described e.g. by van Ravenzwaay et al. (2007),Looser et al., (2005), Weckwerth and Morgenthal (2005), and Wilson et al. (2005). As shown in previous publications, metabolite profiles as indicators of pathway (de) regulations are predetermined by physiological factors like rat strain, gender, circadian rhythm, estrus cycle, as well as by the composition of the diet (Bollard et al., 2001; Dumas et al., 2006; Gavaghan et al., 2000, 2001; Li et al., 2007; Mellert et al., 2011; Plumb et al., 2005; Robosky et al., 2005; Stanley et al., 2005; Strauss et al., 2009; Wang et al., 2006). In toxicology, specific changes of the metabolome in rats often correlate with toxicological modes of action and are discriminable for treatments with defined toxicants, dose and gender (Kamp et al., 2012a,b; Keun, 2006; Mattes et al., 2013; van Ravenzwaay et al., 2007). BASF SE and metanomics GmbH established the metabolomics database MetaMap®Tox with more than 550 reference substances. For all reference compounds the target organs and toxicological modes of action are well documented. All reference substances were administered orally, either via feed or by gavage, in a standardized 28-day study with two dose levels and three blood sampling points (van Ravenzwaay et al., 2007). In combination with the established database, metabolome analysis in rats offers a multitude of potential applications by comparing specific metabolomic changes measured under defined conditions with the available information in the database. Thus, metabolome changes of a new test substance can be compared with metabolome patterns of the reference compounds as well as to predefined patterns of metabolome changes associated with specific modes of action. Metabolome analysis may thus be used as a tool for early detection of toxicological effects and biology based grouping of chemicals (van Ravenzwaay et al., 2012; Ramirez et al., 2013). In addition this analysis provides deeper insight into toxicological modes of action of known test substances (Balcke et al., 2011; Clarke and Haselden, 2008; Mortishire-Smith et al., 2004; Ekman et al., 2006; Montoya et al., 2014), is helpful for the discovery of new biomarkers of effect (van Ravenzwaay et al., 2007) or may be helpful for the assessment of toxicity when organisms are exposed to a combination of toxicants (van Ravenzwaay et al., 2010; Strauss et al., 2012).

In general, observed effects in the plasma metabolome of rats can be attributed to effects of systemic toxicity. However, the route of exposure may modify the toxicity of substances based on different barrier functions or potentially different metabolic first pass effects. For a specific test substance, a comparison of metabolic profiles in plasma, induced by different exposure scenarios, can therefore demonstrate potential administration route-dependent modulations of systemic toxicity. Moreover, we asked the question if the current data base (based on oral administration) can also be used to predict systemic toxicity of compounds administered by inhalation. This extension of the use of the data base is of importance for the identification of systemic toxicity induced by volatile compounds, e.g. some industrial chemicals. While the oral administration reflects a common exposure route for a standard repeated dose toxicity test, inhalative administration reflects more a potential exposure to chemicals at working places. Based on this background, we compared the plasma metabolome of Wistar rats after inhalative exposure versus the metabolome after oral exposure in MetaMap®Tox for selected test substances; to identify similarities, as well as potential differences of effects on the metabolome between the two exposure routes. Investigated test substances were chosen mainly based on their ability to be evaporated under a conditioned supply of air and on the feasibility to generate a homogenous and consistently composed test atmosphere. Additionally, the generated test-substance concentrations had to be analytically verifiable. Based on these preconditions, as well as on known toxicological profiles aniline (A), chloroform (CL), ethylbenzene (EB), 2-methoxyethanol (ME), *N*,*N*-dimethylformamide (DMF) and tetrahydrofurane (THF) were selected for this study.

2. Materials and methods

2.1. Animal welfare and maintenance conditions

The studies were performed in accordance with German Animal Welfare legislation and with the permission of the local authority (approval number 23 177-07/G 08-3-001). The laboratory is AAALAC (Association for Assessment and Accreditation of Laboratory Animal Care International) certified. Wistar (CrI:WI(Han)) rats were supplied by Charles River Laboratories, Sulzfeld, Germany. Animals of the oral dose groups were housed and maintained as described in Kamp et al. (2012b) For the inhalation experiments, animals were housed in groups of 5 animals per cage and gender in H-Temp (PSU) cages, floor area about 2065 cm^2 ($610 \times 435 \times 215$ mm); TECNIPLAST, Germany with wooden gnawing blocks (Typ NGM E-022); Abedd® Lab. and Vet. Service GmbH Vienna, Austria) as enrichment. Animals were maintained in an air-conditioned room at a temperature of 20–24 °C, a relative humidity of 30–70%, and a 12 h light/12 h dark cycle. Before the animals' arrival, the room was completely disinfected and during the study, the floor and walls were cleaned weekly with a solution of 0.1% Incidin® in water. Ground Kliba mouse/rat maintenance diet was supplied by Provimi Kliba SA, Kaiseraugst, Switzerland. The diet and drinking water were available ad libitum (except before blood sampling) and regularly assayed for chemical contaminants and the presence of microorganisms.

2.2. Treatment of animals

Test substances were dosed orally or via inhalation to rats at 2 dose levels for a period of 28 days. All test substances had a purity of ≥99% and A was obtained from Fluka (Buchs, Switzerland), THF from Merck KGaA (Darmstadt, Germany) and CL, EB, ME, DMF from Sigma-Aldrich (Taufkirchen, Germany).

For oral dosing test substances were supplied by feed or gavage (vehicle corn oil or water, see Table 1) to five rats per dose and sex. At the start of the study, the animals were about 10 weeks old.

For inhalative whole body exposures, rats were single-housed in wire cages that were located in a glass-steel inhalation chamber, $V \approx 1.4\ m^3$. For adaptation to the exposure conditions, the animals were exposed to fresh air under comparable flow conditions in whole-body inhalation systems on several days before start of the exposure period. Inhalation atmospheres with the test substances were generated as mixtures with conditioned air (about 50% ± 20% relative humidity, 22 °C ± 2 °C) continuously, as homogeneous as possible and concentrations constancy was controlled. Feed and drinking water was withdrawn from the animals during exposure in the inhalation chambers. Five animals per dose group and sex, aged about 8 weeks were exposed for 6 h a day, 5 days a week, in total for 4 weeks. Control groups, exposed to conditioned air, consisted of 10 animals per sex. The age of the rats of 8 weeks for inhalative exposure is based on the routine study design for