The Journal of Toxicological Sciences (J. Toxicol. Sci.)
Vol.45, No.2, 95-108, 2020

Original Article

Integration of read-across and artificial neural network-based QSAR models for predicting systemic toxicity: A case study for valproic acid

Tomoka Hisaki[1,2], Maki Aiba née Kaneko[1], Morihiko Hirota[1], Masato Matsuoka[2] and Hirokazu Kouzuki[1]

[1]Shiseido Global Innovation Center, 1-2-11 Takashima, Nishi-ku, Yokohama-shi, Kanagawa 220-0011, Japan
[2]Department of Hygiene and Public Health, Tokyo Women's Medical University, 8-1 Kawada-cho, Shinjuku-ku, Tokyo 162-8666, Japan

[Recommended by Takemi Yoshida]

(Received August 13, 2019; Accepted October 3, 2019)

ABSTRACT — We present a systematic, comprehensive and reproducible weight-of-evidence approach for predicting the no-observed-adverse-effect level (NOAEL) for systemic toxicity by using read-across and quantitative structure-activity relationship (QSAR) models to fill gaps in rat repeated-dose and developmental toxicity data. As a case study, we chose valproic acid, a developmental toxicant in humans and animals. High-quality *in vivo* oral rat repeated-dose and developmental toxicity data were available for five and nine analogues, respectively, and showed qualitative consistency, especially for developmental toxicity. Similarity between the target and analogues is readily defined computationally, and data uncertainties associated with the similarities in structural, physico-chemical and toxicological properties, including toxicophores, were low. Uncertainty associated with metabolic similarity is low-to-moderate, largely because the approach was limited to *in silico* prediction to enable systematic and objective data collection. Uncertainty associated with completeness of read-across was reduced by including *in vitro* and *in silico* metabolic data and expanding the experimental animal database. Taking the "worst-case" approach, the smallest NOAEL values among the analogs (i.e., 200 and 100 mg/kg/day for repeated-dose and developmental toxicity, respectively) were read-across to valproic acid. Our previous QSAR models predict repeated-dose NOAEL of 148 (males) and 228 (females) mg/kg/day, and developmental toxicity NOAEL of 390 mg/kg/day for valproic acid. Based on read-across and QSAR, the conservatively predicted NOAEL is 148 mg/kg/day for repeated-dose toxicity, and 100 mg/kg/day for developmental toxicity. Experimental values are 341 mg/kg/day and 100 mg/kg/day, respectively. The present approach appears promising for quantitative and qualitative *in silico* systemic toxicity prediction of untested chemicals.

Key words: Read-across, Valproic acid, Repeated-dose toxicity, Developmental toxicity, No Observed Adverse Effect Level (NOAEL), Weight-of-Evidence (WoE)

INTRODUCTION

Despite the urgent need for alternative, non-animal test methods, the complexity of systemic toxicity makes this endpoint difficult to model. The requirement for well-validated and proven alternative methods to fill gaps in safety data is especially urgent for the cosmetics industry, since the selling of cosmetic products containing substances that have been tested in animals has already been banned in the European Union (EU) by the 7th amendment of the EU cosmetics directive. Nevertheless, the importance of systemic toxicity assessment for cosmetic ingredients has been highlighted in "Notes of Guidance for Testing of Cosmetic Ingredients and Their Safety Evaluation" by the Scientific Committee on Consumer Safety (SCCS) as well as in "Safety Evaluation Guidelines Edition 2014" by the Personal Care Products Council (PCPC), which both strongly recommend calculation of the Margin of

Correspondence: Tomoka Hisaki (E-mail: tomoka.hisaki@shiseido.com)

Safety (MoS). For this purpose, we require no-observed-adverse-effect level (NOAEL) data for repeated-dose, reproductive and developmental toxicities without relying on animal testing. However, because of the multiplicity and complexity of the processes underlying systemic toxicity, no alternative method has yet been brought into general use. Indeed, the embryonic stem cell test (EST) is the only *in vitro* method that has been validated by The European Centre for the Validation of Alternative Methods (ECVAM; Seiler and Spielmann, 2011). However, this method is designed to identify only potential developmental toxicity. Most other *in vitro* methods being developed also have limitations, and a reliable method for quantitative toxicity prediction is still needed.

At present, read-across is one of the few *in silico* methods for predicting systemic toxicity, and is the furthest advanced towards regulatory acceptance. Read-across is a data-gap-filling technique using within-category and analogue approaches for hazard identification and risk assessment (Cronin *et al*., 2013). The guidance from the Organisation for Economic Co-operation and Development (OECD) accepts the extrapolation of measured data to similar untested chemicals for hazard identification and risk assessment through a category or analogue approach (OECD, 2014). In addition, about 75% of European Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) dossiers submitted between 2010 and 2013 contain read-across for at least one endpoint (Ball *et al*., 2016). The read-across method is endorsed by other regulatory authorities worldwide including the Research Institute for Fragrance Materials, the International Council of Chemical Associations (ICCA) and the U.S. Environmental Protection Agency's High Production Volume (HPV) Challenge Program (Bishop *et al*., 2012; Stanton and Kruszewski, 2016).

But, even though read-across has been accepted for years, challenges remain concerning the consistency and robustness of its methodology. Several guidance notes and publications offer guidelines on how to perform, assess and document a read-across study (ECHA, 2008; OECD, 2014; Patlewicz *et al*., 2015; Schultz *et al*., 2015). The importance of two critical steps (i.e. analogue identification and analogue evaluation) is emphasized in all of these documents, but a definitive protocol has not been established. Recently, a series of case studies focusing on repeated-dose toxicity has provided some operative examples of a read-across protocol (Firman *et al*., 2018; Przybylak *et al*., 2017; Schultz *et al*., 2017). However, these protocols are applicable only to a chemical with a simple structure for which analogues can be easily chosen (e.g., analogues with a different number of carbon atoms in the alkyl chain of n-alkanols). Furthermore, there are only a few reports of read-across studies that aimed to make quantitative predictions, such as NOAEL values (Lizarraga *et al*., 2019; Mellor *et al*., 2017; Schultz *et al*., 2017), especially where the data gap involves reproductive or developmental toxicity.

In this study, we propose a systematic, comprehensive and reproducible protocol for read-across that should be applicable to a wide range of chemicals. As a case study, we chose valproic acid (CAS: 99-66-1), or 2-propylpentanoic acid, to illustrate and evaluate the suitability of the method not only for repeated-dose toxicity assessment, but also for other endpoints such as developmental toxicity. Valproic acid is a common developmental toxicant. In humans, exposure during pregnancy causes spina bifida aperta and a typical pattern of minor facial malformations in children (Lammer *et al*., 1987; Robert and Guibaud, 1982). Valproic acid is an embryotoxin in the rat, causing skeletal defects including incomplete ossification, abnormal vertebrae, ribs and craniofacial dysmorphia, cardiovascular defects and hydronephrosis (Binkerd *et al*., 1988). In contrast, it caused only slight hematological alterations, such as increase in blood potassium, decrease in total protein and serum albumin and lymphocytosis, in a 6-month repeated-dose toxicity study in rats (ECHA, 2011a). Experimental studies support the idea that valproic acid causes hepatotoxicity, including changes in serum levels of liver enzymes and low plasma fibrinogen levels, in rats (Tong *et al*., 2005). In the present case study, NOAEL values of 341 and 416 mg/kg/day for male and female rats, respectively, for repeated-dose toxicity, and 100 mg/kg/day for rat developmental toxicity were extrapolated from the ECHA database (ECHA, 2011a); these values are comparable to other reported values.

Here, we present reproducible steps for obtaining valproic acid analogues and for toxicological evaluation. A number of recent guidances and publications emphasize the importance of conducting analogue evaluation in addition to analogue identification (Ball *et al*., 2016; Pradeep *et al*., 2017; Schultz *et al*., 2015). In the present study, analogue identification was done based on a well-known structural similarity calculation method (i.e. Dice), by determining the presence or absence of structural fragments in a fingerprint (Jaworska and Nikolova-Jeliazkova, 2007). However, this method lacks the ability to categorize analogues that are toxicologically similar to the target. Therefore, for analogue evaluation, we considered a range of parameters that could be relevant to the toxicological endpoint in question. We also predicted NOAEL values of repeated-dose and developmental toxicity using a weight-of-evidence (WoE) approach, incorporating

NOAEL values obtained from quantitative structure-activity relationship (QSAR) models.

MATERIALS AND METHODS

Overview of the approach

Figure 1 summarizes the workflow in this study. The read-across approach was adapted from several studies, including Wang *et al*. (2012), OECD (2014) and Schultz *et al*. (2015). First, a set of structurally similar analogues was selected in an objective manner using OECD QSAR Toolbox (version 4.2.). Second, the available animal experimental data was collected systematically. Third, analogues with available toxicity data were used to evaluate the data confidence and uncertainty. Fourth, NOAEL was predicted using QSAR models to provide input for the WoE approach. Finally, predicted NOAELs of repeated-dose and developmental toxicity were established for the target chemical.

Identification of Analogues

A list of possible analogues of valproic acid was acquired using OECD QSAR Toolbox. To expand the number of possible analogues, the compounds listed in all databases under "Human Health Hazards" were defined by the category of structural similarity with the following options: (1) "Dice" for the measure, (2) "Atom centered fragments" for the molecular features, (3) "Hologram" and "Combine all features" for the calculation, (4) "Atom type" and "Hybridization" for the atom characteristics and (5) a threshold of ≥ 80% structural similarity with valproic acid.

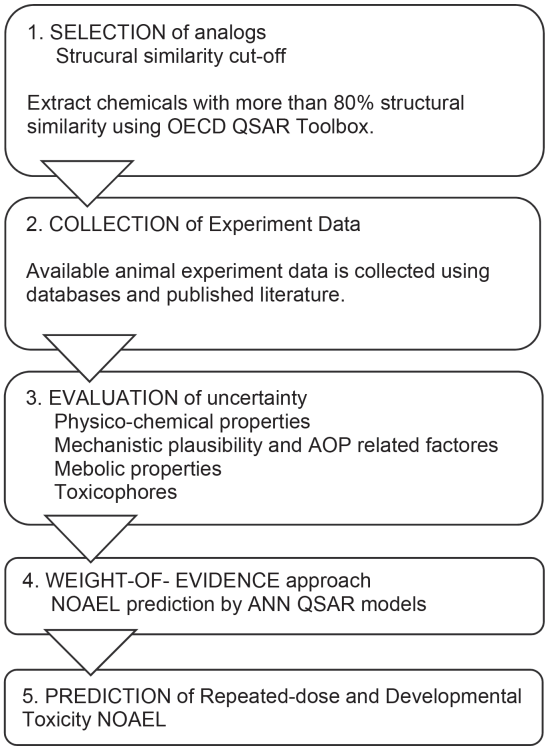


Fig. 1. Overall read-across analysis and Weight-of evidence approach workflow.

Data Collection of Selected Analogues

Toxicity Data

To fill a NOAEL data gap for the target chemical, it is important to obtain as many *in vivo* data of analogues for the endpoint under consideration as possible. Therefore, various sources, including the registration dossier of the European Chemicals Agency (ECHA), ILSI Developmental Toxicity Studies, and published literature were explored in addition to the data already stored in OECD QSAR Toolbox. Moreover, their details and reliability were verified manually by directly accessing the data sources when appropriate. The validity of NOAEL values was checked from the raw data or the full report, when available. However, some databases do not allow access to a full report; in those cases, the NOAEL values suggested by the authors were still adopted as long as they appeared consistent with available data. In this study, for example, the *in vivo* data with reliability of at least 2 (reliable with restrictions) in ECHA's report or the studies in compliance with the Good Laboratory Practice (GLP) standards were accepted.

The endpoint for this case study was repeated-dose and developmental toxicity. These were assessed using data from protocols comparable to OECD Test No. 408: Repeated Dose 90-Day Oral Toxicity Study in Rodents (OECD, 2018a) or OECD Test No. 414: Prenatal Developmental Toxicity Study (OECD, 2018b). Only NOAEL assigned using rats and oral administration were adopted in order to ensure uniformity of data. These values provide a quantitative expression of the analogues' toxicity.

Criteria for category membership

To conduct toxicological evaluation of analogues, criteria related to physico-chemical and molecular properties (Table 3), mechanistic plausibility and adverse outcome pathway (AOP)-related events (Table 4), potential metabolic products (Table 5) and toxicophores or structural alerts (Table 6) were collected for valproic acid and its analogues using US EPA EPI Suite (version 4.1; US EPA, 2012), MOPAC2016 (Stewart, 2016), OECD QSAR Toolbox, and Derek Nexus 6.0.1/Nexus 2.2.1 (Sanderson and Earnshaw, 1991). For hydrophobici-

ty (logKow) in Table 3, experimentally measured values are presented when available. When using Derek Nexus, alerts of all endpoints only for mammals with a prediction of at least equivocal were adopted. Software used to obtain each criterion is shown in the corresponding table.

NOAEL Prediction Using QSAR models

The QSAR models previously developed using by the authors for the prediction of NOAEL for repeated-dose and developmental toxicities (Hisaki *et al*., 2015) were used. These QSAR models were developed using NOAEL of 421 chemicals for repeated-dose toxicity and 156 for developmental toxicity collected from Japan Existing Chemical Database (JECDB; http://dra4.nihs.go.jp/mhlw_data/jsp/SearchPageENG.jsp). Molecular descriptors to predict toxicity (i.e. total energy, heat of formation, gamma average, ionization potential, sum S and sum N for repeated-dose toxicity and total energy, heat of formation, ionization potential and sum N for developmental toxicity), were calculated using the PM3 Hamiltonian of a semi-empirical MO Package (MOPAC2002), and the NOAEL values were predicted using artificial neural network (ANN) models by QwikNet Ver.2.23.

RESULTS

Read-across

Analogue identification

Analogues of valproic acid were identified based on the protocol described previously. OECD QSAR Toolbox initially defined 33 structurally similar compounds with the predefined threshold of ≥ 80% similarity (data not shown); however, analogues with an invalid CAS number, overlapping structure or undefined chemical structure were excluded at this point, since accurate evaluation of analogue validity, which is mainly based on structure, would be difficult. Finally, 23 analogues were accepted as candidates (Table 1).

Compilation of experimental data and NOAEL values of analogues

Repeated-dose and developmental toxicity information for valproic acid and the available analogues are summarized in Table 2.

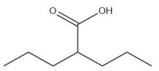
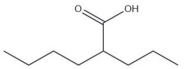
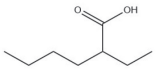
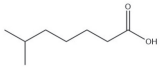
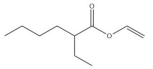
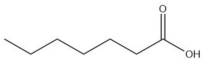
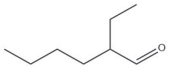
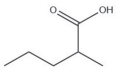
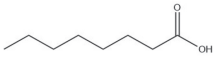
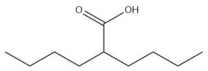
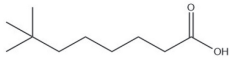
From a repeated-dose toxicity perspective, the preferred test protocol was a 90-day oral repeated-dose toxicity study in rats. While administration route and species

Table 1. Structurally similar analogues of the target chemical.

No.	Name	CAS	SMILES	Structural Similarity*
0	**Valproic Acid**	**99-66-1**	**CCCC(CCC)C(O)=O**	100%
1	2-Propylhexanoic Acid	3274-28-0	CCCCC(CCC)C(O)=O	95.24%
2	2-Ethylhexanoic Acid	149-57-5	CCCCC(CC)C(O)=O	100%
3	6-Methylheptanoic Acid	25103-52-0	CC(C)CCCCC(O)=O	80.00%
4	2-Ethylhexanoic Acid Vinyl Ester	94-04-2	CCCCC(CC)C(=O)OC=C	81.82%
5	Enanthic Acid	111-14-8	CCCCCCC(O)=O	84.21%
6	2-Ethylhexanal	123-05-7	CCCCC(CC)C=O	84.21%
7	2-Methylpentanoic Acid	97-61-0	CCCC(C)C(O)=O	88.89%
8	Octanoic Acid	124-07-2	CCCCCCCC(O)=O	80.00%
9	2-Butylhexanoic Acid	3115-28-4	CCCCC(CCCC)C(O)=O	90.91%
10	2,2-Dimethyloctanoic Acid	26896-20-8	CC(C)(C)CCCCCC(O)=O	81.82%
11	Zinc 2-Ethylhexanoate	85203-81-2	[Zn+2].CCCCC(CC)C([O-])=O	95.24%
12	2-Ethylhexanoyl Chloride	760-67-8	CCCCC(CC)C(Cl)=O	80.00%
13	Methyl 2-Ethylhexanoate	816-19-3	CCCCC(CC)C(=O)OC	85.71%
14	1-Methylcyclohexanecarboxylic Acid	1123-25-7	CC1(CCCCC1)C(O)=O	80.00%
15	2-Ethylbutanoic Acid	88-09-5	CCC(CC)C(O)=O	88.89%
16	2-Methylbutanoic Acid	116-53-0	CCC(C)C(O)=O	82.35%
17	2-Methylbutanoic Acid	600-07-7	CCC(C)C(O)=O	82.35%
18	Sodium 2-Ethylhexanoate	19766-89-3	[Na+].CCCCC(CC)C([O-])=O	95.24%
19	Potassium 2-Ethylhexanoate	3164-85-0	[K+].CCCCC(CC)C([O-])=O	95.24%
20	Propylene Glycol Caprylate	31565-12-5	CC(O)CO.CCCCCCCC(O)=O	80.00%
21	Methyl 2-Ethylhexanoate	816-19-3	CCCCC(CC)C(=O)OC	85.71%
22	3-Ethyl-2-hydroxyheptanoic Acid	63834-30-0	CCCCC(CC)C(O)C(O)=O	81.82%
23	Valpromide	2430-27-5	CCCC(CCC)C(N)=O	80.00%

*Values typically derived from OECD QSAR Toolbox (v4.2.).

Table 2. Summary of repeated-dose and developmental toxicity information for target and available analogues.

No.	Name	CAS	2D Structure	NO(A)EL (mg/kg/day)				
				Repeated Dose Toxicity	Major Findings	Developmental Toxicity	Major Findings	Reference
0	**Valproic Acid**	**99-66-1**		**(M: 341) [a]** **(F: 416) [a]**	**Haematological alterations** **Hepatotoxicity**	**(100) [a]**	**Skeletal defects (reduced ossification, abnormal vertebrae, ribs and craniofacial dysmorphia)** **Visceral defects (cardiovascular abnormalities, hydronephrosis)**	ECHA, 2011a Binkerd *et al.*, 1988 Tong *et al.*, 2005
1	2-Propylhexanoic Acid	3274-28-0		N/A	-	< 900 [b]	Skeletal defects (lumbar ribs) Decreased fetal viability	Narotsky *et al.*, 1994
2	2-Ethylhexanoic Acid	149-57-5		M: 300 F: 300	Decreased body weight gain Reduced feed consumption Haematological alterations Hepatotoxicity	100	Skeletal defects (reduced ossification) Visceral defects (on brain)	ECHA, 2011e
3	6-Methylheptanoic Acid	25103-52-0		N/A	-	800	No significant toxicity observed	Ambroso, 1999
4	2-Ethylhexanoic Acid Vinyl Ester	94-04-2		200 [b]	Increased mortality Haematological alterations	350	Skeletal defects (abnormal vertebrae and ribs) Filamentous tail Reduced foetal body weight	ECHA, 2011b
5	Enanthic Acid	111-14-8		M: 1000 F: 1000	No significant toxicity observed	1000	No significant toxicity observed	ECHA, 2010
6	2-Ethylhexanal	123-05-7		N/A	-	300	Skeletal defects (reduced ossification, abnormal vertebrae and ribs) Visceral defects (on brain, thyroid gland, kidney, and cardiovascular abnormalities) Placental abnormalities Reduced foetal body weight	ECHA, 2011f
7	2-Methylpentanoic Acid	97-61-0		N/A	-	250 [b]	No significant toxicity observed	ECHA, 2018
8	Octanoic Acid	124-07-2		M: 12500 [b] F: 12500 [b]	No significant toxicity observed	1500	No significant toxicity observed	ECHA, 2011c
9	2-Butylhexanoic Acid	3115-28-4		N/A	-	< 1200 [b]	Skeletal defects (lumbar ribs)	Narotsky *et al.*, 1994
10	2,2-Dimethyloctanoic Acid	26896-20-8		M: 700 [b] F: 700 [b]	No significant toxicity observed	N/A	-	ECHA, 2011d

a: The NOAEL value of a target chemical may not be available for real-case.
b: Some discrepancy in the protocol is observed. Refer to "RESULTS - Compilation of experimental data and NOAEL values of analogues" for details.

Table 3. Summary of physico-chemical properties.

No.	Name	Molecular Formula	US EPA EPI Suite (v4.1.)		MOPAC 2016						OECD QSAR Toolbox (v4.2.)	
			Molecular Weight (g/mol)	Log Kow	Total Energy (eV)	Heat of Formation (Kcal/Mol)	Gamma Average (a.u.)	Ionization Potential (EV)	Sum S	Sum N	**Group Count**[b]	Organic functional groups
0	**Valproic Acid**	**C9H18O2**	**144.22**	**2.96** **2.75**[a]	**-1790.6**	**-130.0**	**3894.7**	**10.88**	**0.0**	**0.0**	**2**	**Alkane, branched with tertiary carbon** **Carboxylic acid**
1	2-Propylhexanoic Acid	C9H18O2	158.24	3.45 3.01[a]	-1940.5	-134.7	4451.2	10.88	0.0	0.0	2 (2)	Alkane, branched with tertiary carbon Carboxylic acid
2	2-Ethylhexanoic Acid	C8H16O2	144.22	2.96 2.64[a]	-1790.7	-131.0	3996.2	10.85	0.0	0.0	2 (2)	Alkane, branched with tertiary carbon Carboxylic acid
3	6-Methylheptanoic Acid	C8H16O2	144.22	2.96	-1790.7	-132.0	3596.9	10.93	0.0	0.0	3 (2)	Alkane, branched with tertiary carbon Carboxylic acid Isopropyl
4	2-Ethylhexanoic Acid Vinyl Ester	C10H18O2	170.25	3.6	-2062.3	-104.7	7253.2	9.95	0.0	0.0	3 (1)	Alkane, branched with tertiary carbon Alkene Carboxylic acid ester
5	Enanthic Acid	C7H14O2	130.19	2.54 2.42[a]	-1640.7	-126.4	3279.4	11.07	0.0	0.0	2 (1)	Carboxylic acid Surfactants - Anionic
6	2-Ethylhexanal	C8H16O	128.22	2.71	-1494.5	-69.6	3683.1	9.94	0.0	0.0	2 (1)	Aldehyde Alkane, branched with tertiary carbon
7	2-Methylpentanoic Acid	C6H12O2	116.16	1.98 1.80[a]	-1490.8	-121.4	3093.6	10.94	0.0	0.0	2 (2)	Alkane, branched with tertiary carbon Carboxylic acid
8	Octanoic Acid	C8H16O2	144.22	3.03 3.05[a]	-1790.7	-131.5	3550.5	11.01	0.0	0.0	2 (1)	Carboxylic acid Surfactants - Anionic
9	2-Butylhexanoic Acid	C10H20O2	172.27	3.94 3.20[a]	-2090.4	-138.6	5055.1	10.86	0.0	0.0	2 (2)	Alkane, branched with tertiary carbon Carboxylic acid
10	2,2-Dimethyloctanoic Acid	C10H20O2	172.27	3.83	-2090.6	-141.4	4875.2	10.79	0.0	0.0	1 (0)	Alkane, branched with quaternary carbon

a: Experimentally derived values
b: Number of groups (Number of groups in common with the target)

Table 4. Summary of mechanistic plausibility and adverse outcome pathway (AOP)-related properties.

No.	Name	OECD QSAR Toolbox (v4.2.)			
		Lipinski Rule Oasis	Toxic hazard classification by Cramer	Estrogen Receptor Binding	Retinoic Acid Receptor Binding
0	**Valproic Acid**	**Bioavailable**	**Low (Class I)**	**Non binder, non cyclic structure**	**Not possible to classify according to these rules**
1	2-Propylhexanoic Acid	Bioavailable	Low (Class I)	Non binder, non cyclic structure	Not possible to classify according to these rules
2	2-Ethylhexanoic Acid	Bioavailable	Low (Class I)	Non binder, non cyclic structure	Not possible to classify according to these rules
3	6-Methylheptanoic Acid	Bioavailable	Low (Class I)	Non binder, non cyclic structure	Not possible to classify according to these rules
4	2-Ethylhexanoic Acid Vinyl Ester	Bioavailable	Low (Class I)	Non binder, non cyclic structure	Not possible to classify according to these rules
5	Enanthic Acid	Bioavailable	Low (Class I)	Non binder, non cyclic structure	Not possible to classify according to these rules
6	2-Ethylhexanal	Bioavailable	Low (Class I)	Non binder, non cyclic structure	Not possible to classify according to these rules
7	2-Methylpentanoic Acid	Bioavailable	Low (Class I)	Non binder, non cyclic structure	Not possible to classify according to these rules
8	Octanoic Acid	Bioavailable	Low (Class I)	Non binder, non cyclic structure	Not possible to classify according to these rules
9	2-Butylhexanoic Acid	Bioavailable	Low (Class I)	Non binder, non cyclic structure	Not possible to classify according to these rules
10	2,2-Dimethyloctanoic Acid	Bioavailable	Low (Class I)	Non binder, non cyclic structure	Not possible to classify according to these rules

were limited to oral administration in rats, some variations in test method were accepted as long as they were comparable to the test protocol noted above. Specifically, administration of 2-ethylhexanoic acid vinyl ester was limited to 5 days a week for 2 weeks, but the study was conducted following the GLP regulations with sufficient observations (ECHA, 2011b). The sensitivity of this short exposure study may not be as high as that of a 90-day study, but we decided to still incorporate this data as a source of WoE. Similarly, administration of octanoic acid was limited to 84 days, but this was accepted especially because the administration dose was much higher than the limit dose of 1000 mg/kg/day (ECHA, 2011c). For 2,2-diemethyloctanoic acid, the highest dose in the study initially was 1000 mg/kg/day but this was reduced to 700 mg/kg/day following the second week of exposure. But, since the rats were exposed to at least 700 mg/kg/day throughout, this value was used as the final NOAEL (ECHA, 2011d). There was no marked discrepancy in the study protocols of the other analogues. Accordingly, NOAEL values of repeated-dose toxicity for five analogues were obtained; among them, 2-ethylhexanoic acid vinyl ester showed the lowest value (i.e. 200 mg/kg/day).

Similarly, some differences in the protocol of prenatal developmental toxicity studies were accepted. Specifically, 2-propylhexanoic acid was administered only during GD 8-12 and was tested at only one dose (Narotsky *et al*., 1994). The test for 2-methylpentanoic acid was a screening test and had a small number of dams and reduced scope of examination of malformations (ECHA, 2018). 2-Butylhexanoic acid was also administered at only one dose (Narotsky *et al*., 1994). Even though the sensitivity may not be the same for these studies, we decided to incorporate the data in the WoE approach. The tests for other analogues followed OECD Test No. 414 or employed a very similar protocol. Thus, developmental toxicity NOAEL values for nine analogues were available; among them, the lowest value was 100 mg/kg/day in a study of 2-ethylhexanoic acid.

Analogue evaluation

Similarity of physico-chemical properties

The physico-chemical properties of valproic acid and its analogues are shown in Table 3. Based on OECD QSAR Toolbox, the organic functional groups of valproic acid are “alkane, branched with tertiary carbon” and “carboxylic acid”. All category members, except for 2,2-diemethyloctanoic acid, were placed in both or one of the same organic functional group categories.

Similarity of mechanistic plausibility and adverse outcome pathway (AOP)-related properties

The mechanistic plausibility and AOP-related properties of valproic acid and its analogues are summarized in Table 4. Lipinski’s rule is a useful concept to understand the oral absorption of compounds with drug-like bioactivity (Lipinski *et al*., 2001). Valproic acid and all analogues were determined to be bioavailable according to this rule. The Cramer classification proposes three classes of oral toxicity, whereby substances in Class I have simple structures with effective metabolic pathways, and are less likely to have strong oral toxicity, while substances in Class III have suggestive evidence of toxicity, or lack evidence of limited oral toxicity (Cramer *et al*., 1978; Kalkhof *et al*., 2012). Valproic acid and all the analogues were included in Class I. From the perspective of developmental toxicity, estrogen receptor-binding and retinoic acid receptor-binding affinity were chosen as general properties mechanistically related to the AOP. Here, the target and analogues were not suggested to bind to these receptors.

Similarity of metabolism

It is generally accepted that, in both rat and human, valproic acid is metabolized almost entirely in the liver via glucuronidation, beta oxidation and cytochrome P450-mediated oxidation (Kiang *et al*., 2010; Tong *et al*., 2005). An important CYP-mediated reaction in the metabolic pathway is the formation of 2-propylpent-4-enoic acid (4-ene-VPA), creating a carbon-carbon double bond. Cytochrome P450 enzymes also catalyze the formation of 4-hydroxy-2-propylpentanoic acid (4-OH-VPA), 5-hydroxy-2-propylpentanoic acid (5-OH-VPA) and 3-hydroxy-2-propylpentanoic acid (3-OH-VPA) through hydroxylation. Moreover, glucuronidation of valproic acid occurs to afford valproic acid glucuronide.

To allow systematic evaluation of metabolic similarity, we used an *in silico* simulation model in OECD QSAR Toolbox (Rat liver S9 metabolism simulator). As summarized in Table 5, the simulation model suggested hydroxylation of valproic acid to form 4-OH-VPA (CCCC(CC(C)O)C(O)=O) and 5-OH-VPA (CCCC(CCC=O)C(O)=O), as well as production of unsaturated 4-ene-VPA (CCCC(CC=C)C(O)=O) as metabolites. While all analogues, except 2-ethylhexanal, were predicted to undergo hydroxylation, only 2-propylhexanoic acid and 2-methylpentanoic acid were suggested to produce an unsaturated metabolite.

Similarity of toxicophores

Toxicophore alerts, or structural alerts, for the target substance and analogues are presented in Table 6. This provides information on the similarity of structure frag-

Table 5. Summary of potential metabolic products.

	Target	**Analogues**				
No.	**0**	1	2	3	4	5
Name	**Valproic Acid**	2-Propylhexanoic Acid	2-Ethylhexanioic Acid	6-Methylheptanoic Acid	2-Ethylhexanoic Acid Vinyl Ester	Enanthic Acid
Metabolites	OC(=O)C(CC=C)CC=C OCCCC(CC=C)C(O)=O CC(O)CC(CC=C)C(O)=O CCCC(CC(C)O)C(O)=O (H) CCCC(CC=C)C(O)=O (U) CCCC(CC1CO1)C(O)=O CCCC(CCC(O)=O)C(O)=O CCCC(CCC=O)C(O)=O CCCC(CCCO)C(O)=O (H)	OCCCCC(CC=C)C(O)=O CC(O)CCC(CC=C)C(O)=O CCCC(CCC(C)O)C(O)=O (H) CCCC(CCCC(O)=O)C(O)=O CCCC(CCCC=O)C(O)=O CCCC(CCCCO)C(O)=O (H) CCCC(O)C(CC=C)C(O)=O CCCCC(C(O)C=C)C(O)=O CCCCC(CC(C)O)C(O)=O (H) CCCCC(CC=C)C(O)=O (U) CCCCC(CC1CO1)C(O)=O CCCCC(CCC(O)=O)C(O)=O CCCCC(CCC=O)C(O)=O CCCCC(CCCO)C(O)=O (H)	CCC(CCC(C)O)C(O)=O (H) CCC(CCCC(O)=O)C(O)=O CCC(CCCC=O)C(O)=O CCC(CCCCO)C(O)=O (H) CCCC(O)C(CC)C(O)=O (H) CCCCC(C(C)O)C(O)=O (H) CCCCC(CC(O)=O)C(O)=O CCCCC(CC=O)C(O)=O CCCCC(CCO)C(O)=O (H)	CC(C)(O)CCCCC(O)=O (H) CC(C)CCC(O)=O CC(CCCCC(O)=O)C(O)=O CC(CCCCC(O)=O)C=O CC(CO)CCCCC(O)=O (H) CC(O)=O	OC=C CC(O)=O CC=O CCC(CCC(C)O)C(O)=O CCC(CCCC(O)=O)C(=O)OC=C CCC(CCCC=O)C(=O)OC=C CCC(CCCCO)C(=O)OC=C (H) CCC(CCCCO)C(O)=O CCCC(O)C(CC)C(O)=O CCCCC(C(C)O)C(O)=O CCCCC(CC(O)=O)C(=O)OC=C CCCCC(CC)C(O)=O CCCCC(CC=O)C(=O)OC=C CCCCC(CCO)C(=O)OC=C (H) CCCCC(CCO)C(O)=O	CC(=O)CCCCC(O)=O CC(O)=O CC(O)CCC(O)=O CC(O)CCCCC(O)=O (H) CCCCC(O)=O OC(=O)CCCC=O OC(=O)CCCCCC(O)=O OC(=O)CCCCCC=O OCCCCC(O)=O OCCCCCCC(O)=O (H)
Hydroxylation [a]	2	4	5	2	2	2
Unsaturation [b]	1	1	0	0	0	0

	Analogues				
No.	6	7	8	9	10
Name	2-Ethylhexanal	2-Methylpentanoic Acid	Octanoic Acid	2-Butylhexanoic Acid	2,2-Diemethyloctanoic Acid
	CCCCC(CC)C(O)=O	CC(C(O)C=C)C(O)=O CC(CC(O)CO)C(O)=O CC(CC=C)C(O)=O (U) CC(CC1CO1)C(O)=O CC(CCC(O)=O)C(O)=O CC(CCC=O)C(O)=O CC(CCCO)C(O)=O (H) CC(O)CC(C)C(O)=O (H)	CC(=O)CCCCCC(O)=O CC(O)=O CC(O)CCCC(O)=O CC(O)CCCCCC(O)=O (H) CCCC(O)=O CCCCCC(O)=O OC(=O)CCCCCCC(O)=O OC(=O)CCCCCCC=O OCCCCCCCC(O)=O (H)	CCCCC(C(O)CCC)C(O)=O (H) CCCCC(CCC(C)O)C(O)=O (H) CCCCC(CCCC(O)=O)C(O)=O CCCCC(CCCC=O)C(O)=O CCCCC(CCCCO)C(O)=O (H)	CC(C)(C)CCCC(O)=O CC(C)(CCCCCC(O)=O)C(O)=O CC(C)(CCCCCC(O)=O)C=O CC(C)(CO)CCCCCC(O)=O (H) CC(O)=O
Hydroxylation [a]	0	2	2	3	1
Unsaturation [b]	0	1	0	0	0

Metabolites typically derived from OECD QSAR Toolbox (v4.2.; Rat liver S9 metabolism simulator)
a: The number of hydroxylated metabolites formed by adding a hydroxyl group.
b: The number of unsaturated metabolites formed by creating a CC double bond.
(H): Metabolites in hydroxylated form
(U): Metabolites in unsaturated form

ments related to the toxicological endpoints of interest. For general systemic toxicity, the presence of structural alerts was predicted based on repeated dose (HESS) using OECD QSAR Toolbox and based on toxicity using Derek Nexus. Repeated dose (HESS) gave four alerts for valproic acid, and all of the analogues, except 2-ethylhexanal, gave one or more of the same alerts. Derek Nexus also gave four alerts for valproic acid, and most of the analogues had one or more alerts in common, except for 2-ethylhexanal and 2,2-dimethyloctanoic acid. For developmental toxicity, DART scheme and rtER Expert System in OECD QSAR Toolbox were used. Valproic acid as well as three analogues (2-propylhexanoic acid, 2-ethylhexanioic acid and 2-butylhexanoic acid) had the alert of "Alpha-alkylcarboxylic acid derivatives (22c)" which supports their toxicological similarity. In contrast, rtER Expert System did not generate any structural alerts for the target or its analogues.

Estimation of NOAEL values by read-across method

For data-gap filling of a target NOAEL, a "worst-case" approach was adopted to minimize potential under-estimation of toxicity. Specifically, the smallest NOAEL value among well-trusted analogues was assumed to be the NOAEL of the target for the risk assessment. In this case study, the smallest repeated-dose and developmental toxicity NOAEL values were 200 mg/kg/day for 2-ethylhexanoic acid vinyl ester and 100 mg/kg/day for 2-ethylhexanoic acid respectively (Table 2). As discussed below, these analogues did not show any marked discrepancy from valproic acid in any of the factors assessed, so we considered that it was reasonable to use the NOAEL values of these two chemicals. Therefore, NOAEL values of 200 and 100 mg/kg/day for repeated-dose and developmental toxicity, respectively, were read-across to fill the data gap of valproic acid.

Prediction of NOAEL values by artificial neural network based QSAR models

Values of molecular descriptors of valproic acid calculated using MOPAC2002 were -43531.2 eV for total energy, -139.9 Kcal/Mol for heat of formation, 5488.5 a.u. for gamma average, 254.4 EV for ionization potential, 0 for sum S and 0 for sum N. Results of NOAEL values obtained using QSAR models (Hisaki *et al*., 2015) are presented in Table 8. Repeated-dose toxicity NOAEL values of valproic acid in males and females were predicted to be 148 and 228 mg/kg/day respectively, and developmental toxicity NOAEL was predicted to be 390 mg/kg/day.

DISCUSSION

Structural and toxicological similarity evaluation of analogues

The read-across approach was evaluated considering the level of similarity as well as toxicity of each analogue.

Regarding physico-chemical properties (Table 3), while many analogues had similar molecular weight and logKow to those of valproic acid, some, 2-butylhexanoic acid and 2,2-diemethyloctanoic acid for example, had large values for both properties, which reduces its reliability as an analogue. Molecular-orbital descriptors of total energy, heat of formation, gamma average, ionization potential, sum S and sum N were calculated using MOPAC2016; these descriptors are considered to influence the NOAEL of systemic toxicity (Hisaki *et al*., 2015). Accordingly, analogues with similar descriptor values to the target compound may be more likely to exhibit similar NOAEL values. All analogues were calculated to show descriptor values within the range of 1/2 to 2 times those of valproic acid. Valproic acid and all analogues, except for 2,2-dimethyloctanoic acid, were placed in the same organic functional group categories. This suggests that the systematic analogue identification methodology used in this study could effectively collect chemicals similar to the target, satisfying the criteria for category membership required in some guidelines. Overall, based on the similarity of mechanistic plausibility and AOP-related properties, the analogues are proposed to have low uncertainty of similarity in this regard (Table 4). From Table 5, it can be assumed that 2-propylhexanoic acid and 2-methylpentanoic acid will be metabolized similarly to valproic acid, and so may be more likely to show similar oral toxicity to valproic acid than other analogues. However, it is important to note that the metabolism simulator was not able to predict glucuronidation of valproic acid. Regarding toxicophores, 2-propylhexanoic acid, 2-ethylhexanoic acid and 2-butylhexanoic acid seem to have the most toxicophores in common with valproic acid. In contrast, 2-ethylhexanal did not show a common alert (Table 6). Thus, the reliability of 2-ethylhexanal as an analogue may be questionable.

The results of the toxicological similarity evaluations suggest that 2-propylhexanoic acid has the closest similarity to the target, as it suggested to have a common metabolic pathway (Table 5) and it shows overlapping structural alerts (Table 6). In addition, 2-ethylhexanoic acid, 2-methylpentanoic acid and 2-butylhexanoic acid would also be important analogues, as they appear to be similar to valproic acid in various properties. These analogues

Table 6. Summary of toxicophores.

No.	Name	OECD QSAR Toolbox (v4.2.)				Derek Nexus	
		Alert Count[a]	Repeated dose (HESS)[b]	DART scheme	rtER Expert System	Alert Count[a]	Alert
0	**Valproic Acid**	**4**	**Carboxylic acids (Hepatotoxicity) No rank Ethionine (Hepatotoxicity) Alert Sodium valproate (Renal toxicity) Alert Valproic acid (Hepatotoxicity) Alert**	**Alpha-alkylcarboxylic acid derivatives (22c) Known precedent reproductive and developmental toxic potential**	**No alert found**	**4**	**Carcinogenicity 253 - PLAUSIBLE Hepatotoxicity 546 - PLAUSIBLE Irritation (of the gastrointestinal tract) 253 - PLAUSIBLE Teratogenicity 060 - PROBABLE**
1	2-Propylhexanoic Acid	3 (3)	Carboxylic acids (Hepatotoxicity) No rank Sodium valproate (Renal toxicity) Alert Valproic acid (Hepatotoxicity) Alert	Alpha-alkylcarboxylic acid derivatives (22c) Known precedent reproductive and developmental toxic potential	No alert found	2 (2)	Hepatotoxicity 546 - PLAUSIBLE Teratogenicity 060 - PLAUSIBLE
2	2-Ethylhexanoic Acid	4 (4)	Carboxylic acids (Hepatotoxicity) No rank Ethionine (Hepatotoxicity) Alert Sodium valproate (Renal toxicity) Alert Valproic acid (Hepatotoxicity) Alert	Alpha-alkylcarboxylic acid derivatives (22c) Known precedent reproductive and developmental toxic potential	No alert found	2 (2)	Irritation (of the gastrointestinal tract) 253 - EQUIVOCAL Teratogenicity 060 - PROBABLE
3	6-Methylheptanoic Acid	1 (1)	Carboxylic acids (Hepatotoxicity) No rank	No known precedent reproductive and developmental toxic potential	No alert found	2 (2)	Carcinogenicity 253 - PLAUSIBLE Irritation (of the gastrointestinal tract) 253 - PLAUSIBLE
4	2-Ethylhexanoic Acid Vinyl Ester	2 (2)	Sodium valproate (Renal toxicity) Alert Valproic acid (Hepatotoxicity) Alert	known precedent reproductive and developmental toxic potential	No alert found	2 (1)	Skin sensitisation 425 - EQUIVOCAL Teratogenicity 060 - PLAUSIBLE
5	Enanthic Acid	1 (1)	Carboxylic acids (Hepatotoxicity) No rank	No known precedent reproductive and developmental toxic potential	No alert found	3 (3)	Carcinogenicity 253 - PLAUSIBLE Hepatotoxicity 546 - PLAUSIBLE Irritation (of the gastrointestinal tract) 253 - PLAUSIBLE
6	2-Ethylhexanal	0	Not categorized	No known precedent reproductive and developmental toxic potential	No alert found	4 (0)	Chromosome damage *in vitro* 306 - PLAUSIBLE Mutagenicity *in vitro* 306 - PLAUSIBLE Non-specific genotoxicity *in vitro* 306 - PLAUSIBLE Skin sensitisation 419 - PLAUSIBLE
7	2-Methylpentanoic Acid	3 (3)	Carboxylic acids (Hepatotoxicity) No rank Ethionine (Hepatotoxicity) Alert Valproic acid (Hepatotoxicity) Alert	No known precedent reproductive and developmental toxic potential	No alert found	5 (3)	Carcinogenicity 048 - PLAUSIBLE Carcinogenicity 253 - PLAUSIBLE Hepatotoxicity 546 - PLAUSIBLE Irritation (of the gastrointestinal tract) 048 - PLAUSIBLE Irritation (of the gastrointestinal tract) 253 - PLAUSIBLE
8	Octanoic Acid	1 (1)	Carboxylic acids (Hepatotoxicity) No rank	No known precedent reproductive and developmental toxic potential	No alert found	2 (2)	Carcinogenicity 253 - PLAUSIBLE Irritation (of the gastrointestinal tract) 253 - PLAUSIBLE
9	2-Butylhexanoic Acid	3 (3)	Carboxylic acids (Hepatotoxicity) No rank Sodium valproate (Renal toxicity) Alert Valproic acid (Hepatotoxicity) Alert	Alpha-alkylcarboxylic acid derivatives (22c) Known precedent reproductive and developmental toxic potential	No alert found	1 (1)	Teratogenicity 060 - PLAUSIBLE
10	2,2-Dimethyloctanoic Acid	1 (1)	Carboxylic acids (Hepatotoxicity) No rank	No known precedent reproductive and developmental toxic potential	No alert found	0	No alert found

a: Number of alerts (Number of alerts in common with the target)
b: Category with "No rank" was excluded due to its reliability.

Table 7. Assessment of the read-across in terms of uncertainty, characterized in accordance with published documents (Blackburn and Stuard, 2014; Schultz *et al*., 2015).

Similarity Parameter	Data Uncertainty [a]	Strength of Evidence [b]	Comment
Substance identification and structure classifications	Low	High	Sufficient number (i.e. 10) of analogues were available. All category members have CAS numbers and structural similarity of 80% and above.
Physico-chemical properties and Functional groups	Low	High	Sufficient number of analogues are appropriately similar with respect to key physico-chemical and molecular properties. There is a high degree of consistency between measured and estimated values regarding hydrophobicity (logKow). All category members, except one, belong to the same chemical class/subclass.
Toxicokinetics, AOP and toxicological properties	Low	Medium	Based on model predictions, majority of category members may show similar oral absorption. All belong to the same Cramer classification class, and are not potential binders of recepters studied.
Potential metabolic products	Low to Moderate	Medium	Based on *in silico* metabolic simulation, potential metabolic products of two analogues are highly similar to that of the target.
Toxicophores/ Structural alerts	Low	High	Based on *in silico* profiles, acceptable number of members contain the same toxicophores related to both general systemic and developmental toxicity.

a: Uncertainty associated with underlying information/data used in the exercise.
b: Consistency within the information/data used to support the similarity rational and prediction

Table 8. Predicted NOAEL values by QSAR models (Hisaki *et al*., 2015).

	Predicted NOAEL by QSAR models (mg/kg/day)		
	Repeated Dose Toxicitiy		
Name	Male	Female	Developmental Toxicity
Valproic Acid	148	228	390

had high structural similarity of around 90% (Table 1) as well. In contrast, 2-ethylhexanal or 2,2-diemethyloctanoic acid may have low reliability as analogues, in view of the discrepancy in toxicophores and/or physico-chemical properties.

Statement of uncertainty

A characterization of read-across uncertainty along with summary comments is presented in Table 7. This process was done with reference to published documents, which presented a framework to facilitate the consistent characterization of uncertainty (Blackburn and Stuard, 2014; Schultz *et al*., 2015). Briefly, 10 analogues of valproic acid were available; all have high structural similarity (over 80%) and *in vivo* data of repeated-dose and/or developmental toxicity are available. Some analogues differ in some parameters, but the majority of analogues showed good consistency and therefore, the data uncertainty could still be determined as low. Analogues with limited discrepancies should not necessarily be excluded from the category members for a "worst-case" prediction; the result of analysis would be important for a WoE approach. The strength of evidence for some aspects of toxicokinetics and metabolism is moderate. This is because the analysis was intentionally limited to *in silico* prediction in order to allow systematic and objective data collection. The strength of the evidence could be improved by using a range of *in silico* prediction tools, such as TIMES-SS and Meteor Nexus (Lhasa Limited, Leeds, UK), or conducting *in vitro* metabolic studies.

Safety assessment

Estimation of final NOAEL values

Given the complexity of systemic toxicity endpoints, there is still a need to recognize that individual models lack predictive ability. Currently available *in silico* methods including QSAR models and read-across approaches are only coding for some portion of the overall toxicity mechanisms. However, each method may address a part of the question, and could be used as a tool for a WoE approach. Therefore, NOAEL values were derived based on the results of both read-across and QSAR model methods.

In order to reduce the possibility of under-estimation of target toxicity, a conservative approach was taken at this step as well. The NOAEL values obtained from the two methods were compared and the smallest value was taken as the NOAEL of the target. In this case, NOAEL of repeated-dose toxicity is predicted to be 148 mg/kg/day by the QSAR model (Table 8) and 100 mg/kg/day by the read-across approach (Table 2). Keeping in mind that the value of NOAEL is variable depending on the chosen application dose, test protocols and other factors, the values predicted in this case study are regarded as being in good agreement, without underestimation, with the known NOAEL of valproic acid: 341 mg/kg/day for repeated-dose toxicity and 100 mg/kg/day

for developmental toxicity (ECHA, 2011a).

The QSAR models used in this study were developed using the *in vivo* data of wide range of chemicals, including general chemical substances and drugs, which were collected from JECDB. All the values of input descriptors of valproic acid were within the range of model-constructed datasets. Therefore, we concluded that valproic acid lies within the applicability domain of the QSAR models.

However, it is important to note that many regulatory guidelines (for example ICCR, 2014; SCCS, 2018) maintain a cautious stance regarding the use of QSAR approaches for safety evaluation of substances at the regulatory level. This is mainly because a well-validated QSAR model is not currently available. Limitations include the inability of QSAR models to clearly estimate the toxicity of all types of chemical substances. Nevertheless, the guidelines state that currently available models may provide supporting evidence as part of WoE for safety assessment in internal decision-making. Our ANN-based QSAR models, therefore, were used in this case study with the aim of minimizing the likelihood of underestimation. Incorporation of plural QSAR models based on different algorithms would be useful for enhancing confidence in the validity of QSAR model-based prediction.

Calculation of MoS

From a commercial point of view, calculation of the MoS is a necessary step in systemic toxicity assessment. According to the SCCS notes of guidance (SCCS, 2018), MoS can be obtained by dividing the oral Point of Departure (POD) by the Systemic Exposure Dose (SED). For POD, we can use the predicted smallest NOAEL value derived from the approach reported in the current study (i.e. 100 mg/kg/day in this case). SED depends on the estimated daily exposure to a chemical per kg body weight. In the case of dermal absorption, a previously developed QSAR model can be used to predict human percutaneous absorption rate of a chemical (Atobe *et al*., 2015).

Hazard identification

Hazard identification is also a critical step in systemic toxicity assessment. As regards repeated-dose toxicity, haematological alterations were seen for two (2-ethylhexanioic acid and 2-ethylhexanoic acid vinyl ester) of the five analogues with available *in vivo* data (Table 2), with LOAEL values in the high range of 917-1000 mg/kg/day (ECHA, 2011b, 2011e). Since the number of available repeated-dose toxicity tests was limited, the strength of evidence may not be high, but the possibility of valproic acid to show similar effects should not be denied. Hepatotoxicity of valproic acid is suggested experimentally (Tong *et al*., 2005), but only one analogue (2-ethylhexanoic acid) is reported to affect the liver. Therefore, the analogues do not predict valproic acid's toxicological effect on liver. However, this is not surprising, since hepatotoxicity of valproic acid was not observed in a repeated-dose toxicity study that followed a standard protocol (ECHA, 2011a).

Regarding developmental toxicity, although some protocols varied, skeletal defects were commonly observed for five (2-propylhexanoic acid, 2-ethylhexanioic acid, 2-ethylhexanoic acid vinyl ester, 2-ethylhexanal and 2-butylhexanoic acid) of the nine analogues with available *in vivo* data (Table 2). The major effects involved ribs and vertebrae, accompanied by reduced ossification, with the lowest-observed-adverse-effect level (LOAEL) in the range of 250-797 mg/kg/day (ECHA, 2011b, 2011e, 2011f; Narotsky *et al*., 1994). From the preliminary investigation of available studies of analogues, we concluded that the analogues enabled prediction of valproic acid's skeletal effect.

In summary, when data is available for a sufficient number of analogues, read-across can be useful to predict the likely target organ or major fetal toxicity of a chemical of interest. The facts that several analogues identified in the read-across approach exhibited developmental toxicity in rat studies and that some structural alerts related to teratogenicity were detected for valproic acid (Table 6) increases the confidence that the target possesses a developmental effect.

Moreover, developmental toxicity is only detected at doses equivalent to or higher than the repeated-dose toxicity (Laufersweiler *et al*., 2012; van Ravenzwaay *et al*., 2017) in general. In other words, when a developmental effect is observed at a dose at which general toxicity is not seen, that chemical is likely to be a strong developmental toxicant. In this case study, the NOAEL for developmental toxicity was smaller than that for repeated-dose toxicity and therefore, a developmental toxicity hazard of the target was suggested.

In conclusions, the aim of this study was to investigate a WoE approach for predicting systemic toxicity NOAEL, with valproic acid as a case study, using read-across and QSAR model predictions of the repeated-dose and developmental toxicity. This study focused on establishing a simple and systematic approach using widely available software tools to minimize subjective judgement. The initial step of analogue collection was based solely on mathematical structural similarity calculated with OECD QSAR Toolbox software. This greatly extends the appli-

cability of read-across assessment to wide range of target chemicals, as it does not require empirical selection of analogues. Although some toxicologically differing analogues may be included, the results of the present case study indicate that the structure-based read-across method is an excellent tool for contributing to the judgement of systemic toxicity.

In an effort to minimize the underestimation of NOAEL, we employed a WoE approach, integrating another type of computational method, QSAR models, which contribute to the same goal (i.e. prediction of NOAEL) with a conceptually different scheme. The study further indicates that the proposed method can identify developmental toxicity hazards.

By modifying the categories to be defined in the read-across approach and the tools to be used in WoE, it should also be possible to apply this approach to other toxicity endpoints, including reproductive toxicity. Expansion of the experimental animal database would also be useful for enhancing confidence in the validity of read-across. It would also appear feasible to integrate additional *in silico* prediction tools or *in vitro* tests to reduce the underlying uncertainty, especially in the category of metabolic similarity. Some *in vitro* studies on the prediction of hazards related to systemic toxicity endpoints, such as hepatotoxicity (e.g. Susukida *et al*., 2016) or developmental toxicity (e.g. Le Coz *et al*., 2015), are available, and should serve to increase the accuracy of hazard identification.

Conflict of interest---- The authors declare that there is no conflict of interest.

REFERENCES

Atobe, T., Mori, M., Yamashita, F., Hashida, M. and Kouzuki, H. (2015): Artificial neural network analysis for predicting human percutaneous absorption taking account of vehicle properties. J. Toxicol. Sci., **40**, 277-294.

Ball, N., Cronin, M.T., Shen, J., Blackburn, K., Booth, E.D., Bouhifd, M., Donley, E., Egnash, L., Hastings, C., Juberg, D.R., Kleensang, A., Kleinstreuer, N., Kroese, E.D., Lee, A.C., Luechtefeld, T., Maertens, A., Marty, S., Naciff, J.M., Palmer, J., Pamies, D., Penman, M., Richarz, A.N., Russo, D.P., Stuard, S.B., Patlewicz, G., van Ravenzwaay, B., Wu, S., Zhu, H. and Hartung, T. (2016): Toward good read-across practice (GRAP) guidance. ALTEX, **33**, 149-166.

Binkerd, P.E., Rowland, J.M., Nau, H. and Hendrickx, A.G. (1988): Evaluation of valproic acid (VPA) developmental toxicity and pharmacokinetics in Sprague-Dawley rats. Fundam. Appl. Toxicol., **11**, 485-493.

Bishop, P.L., Manuppello, J.R., Willett, C.E. and Sandler, J.T. (2012): Animal use and lessons learned in the U.S. High Production Volume Chemicals Challenge Program. Environ. Health Perspect., **120**, 1631-1639.

Blackburn, K. and Stuard, S.B. (2014): A framework to facilitate consistent characterization of read across uncertainty. Regul. Toxicol. Pharmacol., **68**, 353-362.

Cramer, G.M., Ford, R.A. and Hall, R.L. (1978): Estimation of toxic hazard--a decision tree approach. Food Cosmet. Toxicol., **16**, 255-276.

Cronin, M.T., Madden, J.C., Enoch, S.J. and Roberts, D.W. (2013): Chemical Toxicity Prediction, Issues in Toxicology. Royal Society of Chemistry, Cambridge.

ECHA. (2018): 2-methylvaleric acid [WWW Document]. URL https://echa.europa.eu/registration-dossier/-/registered-dossier/23844 (accessed 7.15.19).

ECHA. (2011a): 2-propylvaleric acid [WWW Document]. URL https://echa.europa.eu/registration-dossier/-/registered-dossier/14340 (accessed 7.15.19).

ECHA. (2011b): Vinyl 2-ethylhexanoate [WWW Document]. URL https://echa.europa.eu/registration-dossier/-/registered-dossier/13394 (accessed 7.15.19).

ECHA. (2011c): Octanoic acid [WWW Document]. URL https://echa.europa.eu/registration-dossier/-/registered-dossier/15370 (accessed 7.15.19).

ECHA. (2011d): Neodecanoic acid [WWW Document]. URL https://echa.europa.eu/registration-dossier/-/registered-dossier/15692 (accessed 7.15.19).

ECHA. (2011e): 2-ethylhexanoic acid [WWW Document]. URL https://echa.europa.eu/registration-dossier/-/registered-dossier/14246 (accessed 7.15.19).

ECHA. (2011f): 2-ethylhexanal [WWW Document]. URL https://echa.europa.eu/registration-dossier/-/registered-dossier/13822 (accessed 7.15.19).

ECHA. (2010): Heptanoic acid [WWW Document]. URL https://echa.europa.eu/registration-dossier/-/registered-dossier/15829 (accessed 7.15.19).

ECHA. (2008): Guidance on information requirements and chemical safety assessment Chapter R.6: QSARs and grouping of chemicals.

Firman, J.W., Patel, A., Date, M., Cronin, M.T. and Schultz, T.W. (2018): Read-across of 90-day rodent repeated-dose toxicity: A case study for selected simple aryl alcohol alkyl carboxylic acid esters. Comput. Toxicol., **7**, 1-8.

Hisaki, T., Aiba née Kaneko, M., Yamaguchi, M., Sasa, H. and Kouzuki, H. (2015): Development of QSAR models using artificial neural network analysis for risk assessment of repeated-dose, reproductive, and developmental toxicities of cosmetic ingredients. J. Toxicol. Sci., **40**, 163-180.

ICCR. (2014): *In silico* Approaches for Safety Assessment of Cosmetic Ingredients, A report for the International Cooperation on Cosmetics Regulation.

Jaworska, J. and Nikolova-Jeliazkova, N. (2007): How can structural similarity analysis help in category formation? SAR QSAR Environ. Res., **18**, 195-207.

Kalkhof, H., Herzler, M., Stahlmann, R. and Gundert-Remy, U. (2012): Threshold of toxicological concern values for non-genotoxic effects in industrial chemicals: re-evaluation of the Cramer classification. Arch. Toxicol., **86**, 17-25.

Kiang, T.K., Teng, X.W., Karagiozov, S., Surendradoss, J., Chang, T.K. and Abbott, F.S. (2010): Role of oxidative metabolism in the effect of valproic acid on markers of cell viability, necrosis, and oxidative stress in sandwich-cultured rat hepatocytes. Toxicol. Sci., **118**, 501-509.

Lammer, E.J., Sever, L.E. and Oakley, G.P. Jr. (1987): Teratogen update: valproic acid. Teratology, **35**, 465-473.

Laufersweiler, M.C., Gadagbui, B., Baskerville-Abraham, I.M.,

Maier, A., Willis, A., Scialli, A.R., Carr, G.J., Felter, S.P., Blackburn, K. and Daston, G. (2012): Correlation of chemical structure with reproductive and developmental toxicity as it relates to the use of the threshold of toxicological concern. Regul. Toxicol. Pharmacol., **62**, 160-182.

Le Coz, F., Suzuki, N., Nagahori, H., Omori, T. and Saito, K. (2015): Hand1-Luc embryonic stem cell test (Hand1-Luc EST): a novel rapid and highly reproducible *in vitro* test for embryotoxicity by measuring cytotoxicity and differentiation toxicity using engineered mouse ES cells. J. Toxicol. Sci., **40**, 251-261.

Lipinski, C.A., Lombardo, F., Dominy, B.W. and Feeney, P.J. (2001): Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv. Drug Deliv. Rev., **46**, 3-26.

Lizarraga, L.E., Dean, J.L., Kaiser, J.P., Wesselkamper, S.C., Lambert, J.C. and Zhao, Q.J. (2019): A case study on the application of an expert-driven read-across approach in support of quantitative risk assessment of p,p′-dichlorodiphenyldichloroethane. Regul. Toxicol. Pharmacol., **103**, 301-313.

Mellor, C.L., Schultz, T.W., Przybylak, K.R., Richarz, A.N., Bradbury, S.P. and Cronin, M.T. (2017): Read-across for rat oral gavage repeated-dose toxicity for short-chain mono-alkylphenols: A case study. Comput. Toxicol., **2**, 1-11.

Narotsky, M.G., Francis, E.Z. and Kavlock, R.J. (1994): Developmental toxicity and structure-activity relationships of aliphatic acids, including dose-response assessment of valproic acid in mice and rats. Fundam. Appl. Toxicol., **22**, 251-265.

OECD. (2018a): Test No. 408: Repeated Dose 90-Day Oral Toxicity Study in Rodents, OECD Guidelines for the Testing of Chemicals, Section 4. OECD Publishing, Paris.

OECD. (2018b): Test No. 414: Prenatal Developmental Toxicity Study, OECD Guidelines for the Testing of Chemicals, Section 4. OECD Publishing, Paris.

OECD. (2014): Guidance on Grouping of Chemicals, Series on Testing & Assessment, No. 194, Second Edi. ed. Paris.

Patlewicz, G., Ball, N., Boogaard, P.J., Becker, R.A. and Hubesch, B. (2015): Building scientific confidence in the development and evaluation of read-across. Regul. Toxicol. Pharmacol., **72**, 117-133.

Pradeep, P., Mansouri, K., Patlewicz, G. and Judson, R. (2017): A systematic evaluation of analogs and automated read-across prediction of estrogenicity: A case study using hindered phenols. Comput Toxicol, **4**, 22-30.

Przybylak, K.R., Schultz, T.W., Richarz, A.-N., Mellor, C.L., Escher, S.E. and Cronin, M.T. (2017): Read-across of 90-day rat oral repeated-dose toxicity: A case study for selected β-olefinic alcohols. Comput. Toxicol., **1**, 22-32.

Robert, E. and Guibaud, P. (1982): Maternal valproic acid and congenital neural tube defects. Lancet, **2**, 937.

Sanderson, D.M. and Earnshaw, C.G. (1991): Computer prediction of possible toxic action from chemical structure; the DEREK system. Hum. Exp. Toxicol., **10**, 261-273.

SCCS. (2018): The SCCS's Notes of Guidance for the Testing of Cosmetic Ingredients and Their Safety Evaluation, 10th Revision, October 2018.

Schultz, T.W., Amcoff, P., Berggren, E., Gautier, F., Klaric, M., Knight, D.J., Mahony, C., Schwarz, M., White, A. and Cronin, M.T. (2015): A strategy for structuring and reporting a read-across prediction of toxicity. Regul. Toxicol. Pharmacol., **72**, 586-601.

Schultz, T.W., Przybylak, K.R., Richarz, A.N., Mellor, C.L., Escher, S.E., Bradbury, S.P. and Cronin, M.T. (2017): Read-across of 90-day rat oral repeated-dose toxicity: A case study for selected n-alkanols. Comput. Toxicol., **2**, 12-19.

Seiler, A.E. and Spielmann, H. (2011): The validated embryonic stem cell test to predict embryotoxicity *in vitro.* Nat. Protoc., **6**, 961-978.

Stanton, K. and Kruszewski, F.H. (2016): Quantifying the benefits of using read-across and *in silico* techniques to fulfill hazard data requirements for chemical categories. Regul. Toxicol. Pharmacol., **81**, 250-259.

Stewart, J.P. (2016): Stewart computational chemistry, MOPAC2016.

Susukida, T., Sekine, S., Nozaki, M., Tokizono, M., Oizumi, K., Horie, T. and Ito, K. (2016): Establishment of a Drug-Induced, Bile Acid-Dependent Hepatotoxicity Model Using HepaRG Cells. J. Pharm. Sci., **105**, 1550-1560.

Tong, V., Teng, X.W., Chang, T.K. and Abbott, F.S. (2005): Valproic acid I: time course of lipid peroxidation biomarkers, liver toxicity, and valproic acid metabolite levels in rats. Toxicol. Sci., **86**, 427-435.

US EPA (2012): Estimation Programs Interface SuiteTM (EPI Suite) for Microsoft® Windows.

van Ravenzwaay, B., Jiang, X., Luechtefeld, T. and Hartung, T. (2017): The Threshold of Toxicological Concern for prenatal developmental toxicity in rats and rabbits. Regul. Toxicol. Pharmacol., **88**, 157-172.

Wang, N.C., Jay Zhao, Q., Wesselkamper, S.C., Lambert, J.C., Petersen, D. and Hess-Wilson, J.K. (2012): Application of computational toxicological approaches in human health risk assessment. I. A tiered surrogate approach. Regul. Toxicol. Pharmacol., **63**, 10-19.