Comparison of points of departure between subchronic and chronic toxicity studies on food additives, food contaminants and natural food constituents

Sabine Guth^{a,2}, Angelika Roth^{a,2}, Barbara Engeli^b, Dirk W. Lachenmeier^c, Alexander T. Cartus^d, Stephanie Hüser^a, Matthias Baum^e, Patrick Diel^f, Gerhard Eisenbrand^g, Jan G. Hengstler^a, Hans-Ulrich Humpf^h, Hans-Georg Joostⁱ, Alfonso Lampen^j, Marcel Leist^k, Doris Marko^l, Pablo Steinberg^m, Angela Mally^{n,1}, Jürg A. Zarn^{b,1,*}

^a Leibniz Research Centre for Working Environment and Human Factors (IfAdo), Ardeystr. 67, 44139, Dortmund, Germany

^b Eidgenössisches Department des Inneren EDI, Bundesamt für Lebensmittelsicherheit und Veterinärwesen BLV, Schwarzenburgstrasse 155, 3003, Bern, Switzerland

^c Chemisches und Veterinäruntersuchungsamt Karlsruhe, Weißenburger Str. 3, 76187, Karlsruhe, Germany

^d University of Kaiserslautern, Food Chemistry and Toxicology, Erwin-Schrodinger-Strasse 52, 67663, Kaiserslautern, Germany

^e Solenis Germany Industries GmbH, Theodor-Heuss-Anlage 12, 68165, Mannheim, Germany

^f Department of Molecular and Cellular Sports Medicine, Institute of Cardiovascular Research and Sports Medicine, German Sport University Cologne, Am Sportpark Müngersdorf 6, 50933, Cologne, Germany

^g Kühler Grund 48/1, 69126, Heidelberg, Germany

^h Institute of Food Chemistry, Westfälische Wilhelms-Universität Münster, Corrensstrasse 45, 48149, Münster, Germany

ⁱ Department of Experimental Diabetology, German Institute of Human Nutrition (DIfE), Arthur-Scheunert-Allee 114-116, 14558, Nuthetal, Germany

^j Department of Food Safety, Bundesinstitut für Risikobewertung (BfR), Max-Dohrn-Strasse 8-10, 10589, Berlin, Germany

^k In Vitro Toxicology and Biomedicine, Department inaugurated by the Doerenkamp-Zbinden Foundation, University of Konstanz, Box 657, 78457, Konstanz, Germany

^l Department of Food Chemistry and Toxicology, Faculty of Chemistry, University of Vienna, Waehringer Str. 38, 1090 Vienna, Austria

^m Max Rubner-Institut, Federal Research Institute of Nutrition and Food, Haid-und-Neu-Str. 9, 76131, Karlsruhe, Germany

ⁿ Department of Toxicology, University of Würzburg, Versbacher Str. 9, 97078, Würzburg, Germany

ARTICLE INFO

Keywords:

Subchronic toxicity studies
Chronic toxicity studies
Extrapolation factor
NOAEL
Point of departure
Food constituents

ABSTRACT

It was generally accepted as a default assumption that No-Observed-Adverse-Effect Levels (NOAELs) or Lowest-Observed-Adverse-Effect Levels (LOAELs) in long-term toxicity studies are lower than in short-term ones, i.e. the toxic potency increases with prolonged exposure duration. Recent studies on pesticides and industrial chemicals reported that subacute, subchronic or chronic NOAELs/LOAELs are similar when study design factors are appropriately considered. We investigated whether these findings also apply to certain food constituents. After reviewing subchronic and chronic toxicity studies on more than 100 compounds, a total of 32 compounds could be included in the analysis. Geometric mean (GM) values of subchronic vs. chronic NOAEL or LOAEL ratios ranged from 1.0 to 2.0, with a geometric standard deviation from 2.2 to 4.2, which is consistent with data reported in the literature. While for many of the investigated compounds the ratio is around 1 – suggesting that health-based guidance values could appropriately be derived from subchronic toxicity studies – our study also identified some substances with higher ratios leading to a GM of around 2. The EFSA Scientific Committee suggested to apply an uncertainty factor of 2 to extrapolate from subchronic to chronic studies and, as a precautionary approach, we concur with this suggestion.

Abbreviations: BMD, bench mark dose; BMDL, bench mark dose lower confidence limit; c, chronic; DEHP, di-(2-ethylhexyl) phthalate; DS, dose spacing; EFSA, European Food Safety Authority; GM, geometric mean; GSD, geometric standard deviation; JECFA, Joint FAO/WHO Expert Committee on Food Additives; LOAEL, Lowest-Observed-Adverse-Effect Level; NOAEL, No-Observed-Adverse-Effect Level; NTP, US National Toxicology Program; sa, subacute; sc, subchronic.

* Corresponding author.

E-mail addresses: Guth@ifado.de (S. Guth), Roth@ifado.de (A. Roth), barbara.engeli@blv.admin.ch (B. Engeli), Dirk.Lachenmeier@cvaaka.bwl.de (D.W. Lachenmeier), cartus@chemie.uni-kl.de (A.T. Cartus), mbaum@solenis.com (M. Baum), diel@dshs-koeln.de (P. Diel), geisenbra@googlemail.com (G. Eisenbrand), Hengstler@ifado.de (J.G. Hengstler), humpf@uni-muenster.de (H.-U. Humpf), joost@dife.de (H.-G. Joost), alfonso.lampen@bfr.bund.de (A. Lampen), marcel.leist@uni-konstanz.de (M. Leist), doris.marko@univie.ac.at (D. Marko), pablo.steinberg@mri.bund.de (P. Steinberg), mally@toxi.uni-wuerzburg.de (A. Mally), juerg.zarn@blv.admin.ch (J.A. Zarn).

¹ Co-senior authors.

² These authors contributed equally to this publication.

<https://doi.org/10.1016/j.fct.2020.111784>

Received 14 July 2020; Received in revised form 10 September 2020; Accepted 16 September 2020

Available online 28 September 2020

0278-6915/© 2020 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

1. Introduction

It has been generally assumed that the toxic potency increases with increasing exposure duration and that, consequently, No-Observed-Adverse-Effect Levels (NOAELs) or Lowest-Observed-Adverse-Effect Levels (LOAELs) derived from long-term (chronic) toxicity studies (NOAEL_c/LOAEL_c) are lower than NOAELs/LOAELs derived from short-term (subacute, subchronic) toxicity studies (NOAEL_{sa}/LOAEL_{sa}, NOAEL_{sc}/LOAEL_{sc}). In the absence of long-term studies, application of extrapolation factors to NOAELs/LOAELs derived from short-term studies may be used for risk assessment in certain legislations. Various studies suggested factors in the range of 1.2 to about 5 to extrapolate from subacute (28-day) or subchronic (90-day) to chronic (≥ 1 year) toxicity studies³ (Batke et al., 2011; Bitsch et al., 2006; Bokkers and Slob, 2005; Doe et al., 2006; Escher et al., 2020; Groeneveld et al., 2004; Kramer et al., 1996; Pieters et al., 1998; Pohl et al., 2010; Woutersen et al., 1984; Zarn et al. 2010, 2011). The European Food Safety Authority (EFSA) Scientific Committee concluded that the extrapolation from a subchronic to a chronic study duration may be performed by application of an uncertainty factor of 2, provided that the 90-day study used to extrapolate is of adequate quality (e.g. similar parameters were investigated as usually carried out in chronic studies) (EFSA, 2012b). The EFSA Scientific Committee was not in a position to propose default values to extrapolate from a subacute to a chronic study duration because there was less confidence that toxic effects identified in 90-day or chronic studies would be detected in studies of shorter duration (EFSA, 2012b). Therefore, it was recommended to consider an extrapolation from a subacute to a chronic study duration on a case-by-case basis (EFSA, 2012b).

Previous studies have examined the influence of exposure duration on reference points (also termed points of departure, usually expressed as NOAEL or bench mark doses [BMD]) in mice, rats and dogs (summarized in Zarn and O'Brien (2018)). For pesticides, in particular, a large database of subacute, subchronic and chronic toxicity studies is available and, thus, the analysis of these data may deliver the most reliable results. For a substantial number of the pesticides, regardless of the chemical structure/class, the subacute, subchronic or chronic NOAELs or LOAELs in rodents (rats and mice) are similar when study design factors (e.g. animal number, dose spacing, dose decrement in feeding studies) are taken into account (Zarn et al., 2011; Zarn et al., 2013; Zarn and O'Brien, 2018). These findings hold also true for dogs. Several authors reported that an extension of the exposure duration from subchronic (13 weeks) to chronic scenarios (52–104 weeks) has no significant influence on the NOAEL (Box and Spielmann, 2005; Dellarco et al., 2010; Kobel et al., 2010; Spielmann and Gerbracht, 2001; Zarn et al., 2010). In addition, similar findings have been reported for industrial chemicals (e.g. Batke et al., 2011; Escher et al., 2020).

Extended studies with pesticides confirmed the findings described above for an even wider range of exposure durations, including developmental and reproductive toxicity studies (Zarn and O'Brien, 2018). For reproductive toxicity studies, two-thirds of the parental subchronic NOAELs and LOAELs were equal to or even lower than the corresponding values from chronic studies (Zarn and O'Brien, 2018). Overall, no statistically significant differences in NOAELs for pesticides derived from developmental, subacute, subchronic, reproductive and chronic toxicity studies covering exposure durations between 2 and 104 weeks were observed (Zarn and O'Brien, 2018). This is in line with landmark studies by Janer and colleagues that found that reference values derived from 2-generation studies (according to OECD TG 416) in up to 176 cases were not lower than the values that would have been obtained after the first generation or after a subchronic study (Janer et al. 2007a, 2007b).

³ From now on, the subacute, subchronic or chronic toxicity studies will be simply named subacute, subchronic or chronic studies.

In contrast to the extensive regulatory data requirements for pesticides, the tiered approach for toxicity testing of food additives adopted by EFSA (EFSA, 2012a) states that results from repeated dose 90-day oral toxicity studies conducted in Tier 1 “can be used to identify a BMDL or a NOAEL for food additives”. For the safety assessment of natural food constituents or food contaminants not intentionally added to food, long-term studies may not be readily available. Therefore, we were interested in determining if, as in the case of pesticides, the NOAELs for food constituents, either naturally occurring or added intentionally to food such as food additives, natural constituents as well as certain contaminants, are related or unrelated to exposure duration. To this end, we analysed the influence of exposure duration on toxic potency by comparing NOAELs (and LOAELs) derived from subchronic and chronic oral toxicity studies in mice and rats. The role of exposure duration on NOAELs and LOAELs is discussed in the context of the available literature. New insights regarding this topic may have an impact on the future planning of animal experiments in the context of safety assessment of food additives or contaminants and animal welfare.

2. Material and methods

2.1. Identification of study pairs

Publicly available oral toxicity data on food additives, food contaminants or other food ingredients were extracted from safety evaluations conducted by EFSA, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the US National Toxicology Program (NTP). The literature search was conducted in January 2019 by screening the EFSA, JECFA and NTP websites.

We analysed more than 100 evaluations: all EFSA evaluations and/or JECFA evaluations of food colouring agents and sweeteners as well as those of selected preservatives/antioxidants and selected NTP reports to identify study pairs of subchronic and chronic NOAELs/LOAELs in the same species (rats, mice). EFSA reassessed the safety of 41 food colouring agents from 2009 to 2016, by considering all available scientific studies and data. Nineteen sweeteners are authorized as food additives in the EU. The evaluations of most of the sweeteners date back to the 1980s, sometimes relying on unpublished industrial studies, but EFSA started a call for technical and toxicological data with the 30th of June 2018 as deadline for submission. Only a few sweeteners were recently re-evaluated, so that the data base to identify study pairs of subchronic and chronic NOAELs/LOAELs in the same species was limited.

Only studies conducted in rats and mice by the oral administration route (i.e. feeding and gavage studies) were considered whereby gavage and feeding studies were only compared among one another. Genotoxic compounds were excluded from the evaluation because it is assumed that there is no toxicological threshold for compounds being both genotoxic and carcinogenic. Bioaccumulating compounds were also excluded from the evaluation because their adverse effects only become apparent when the body burden has reached a toxicological threshold. Studies on extracts or compound mixtures not further specified were not considered because it is assumed that for the various constituents different toxicological thresholds might exist. As previously described by Zarn et al. (2011), the data extracted for each study included the chemical class of the compound, the animal species and strain, the number of animals per group and sex, the study duration, and all dose levels expressed as feed concentrations (ppm) and/or doses (mg/kg bodyweight (bw) per day), as calculated and reported by the evaluating authority (see “Supplemental material”). Since NOAELs and LOAELs are usually not included in the NTP reports, these values were derived by the authors by identifying the lowest dose providing an adverse effect. For example, effects on body and organ weight or histopathological changes were considered as adverse. Studies with an exposure duration of 9–19 weeks were classified as subchronic studies and those with an exposure duration longer than 60 weeks as chronic studies.

Studies including an experimentally based NOAEL as well as a

LOAEL were considered most informative because they provide the best approximation to the true threshold. In the absence of a NOAEL, a surrogate NOAEL was derived by dividing the LOAEL derived from the study by a factor of 3. While according to EFSA guidance (EFSA, 2012b), the size of this uncertainty factor should be determined on a case-by-case basis when using the LOAEL approach, ECHA suggests to apply an assessment factor of 3 in the majority of cases (ECHA, 2012). In a recent study (Braakhuis et al., 2018), this approach was also used. Studies in which no effects were observed (i.e. no LOAEL could be identified) were excluded from the analysis, since any assumption regarding the NOAEL would be speculative.

The life-stage-dependent dose decrement was previously identified as a major factor leading to apparent differences in NOAELs and/or LOAELs derived from feeding studies with different exposure durations (Zarn et al., 2013). During the course of a feeding study, the administered dose steadily decreases by quite substantial factors. In rodent feeding studies, the food consumption per animal essentially remains unchanged after the subacute phase and, hence, the body weight-related dose decreases due to the increased body weight of the animals. It was reported that the mean dose across the chronic phase is 1.6-fold lower than the mean dose across the subchronic phase of a study (Zarn et al., 2013; Zarn and O'Brien, 2018). To account for this dose decrement in feeding studies, in the present study a factor of 1.6 was applied to NOAELs and LOAELs of subchronic feeding studies if the NOAEL was not a surrogate NOAEL (i.e. 3 cases each in mouse and rat studies).

Overall, 32 substances were included in the analysis: amaranth (dye), anthraquinone, azorubine, butylhydroquinone, butylhydroxyanisole, *trans*-cinnamaldehyde, citral, curcumin, di-(2-ethylhexyl) phthalate (DEHP), erythrosine, eugenol, fumonisin B₁, geranyl acetate, 2,4-hexadienal, 5-hydroxymethyl-2-furfural, indole-3-carbinole, isoeugenol, *D*-limonene, malonaldehyde, melamine, 2-methylimidazole, 4-methylimidazole, β -myrcene, neotame, ochratoxin A, Ponceau 4R, pulegone, Red 2G, sucralose, α,β -thujone, urotropin and zeaxenone. Ten of these substances are authorized food additives: 5 food colouring agents (amaranth, azorubine, curcumin, erythrosine, Ponceau 4R), 2 sweeteners (neotame, sucralose), and 3 preservatives (butylhydroquinone, butylhydroxyanisole, urotropin). One compound is a food colouring agent no longer authorized in the EU (Red 2G). The remaining 21 substances, which might occur in food (natural ingredients, natural flavouring substances, contaminants), were tested by NTP: anthraquinone, *trans*-cinnamaldehyde (microencapsulated), citral (microencapsulated), DEHP, eugenol, fumonisin B₁, geranyl acetate (71% with 29% citronellol acetate), 2,4-hexadienal, 5-hydroxymethyl-2-furfural, indole-3-carbinole, isoeugenol, *D*-limonene, malonaldehyde (sodium salt), melamine, 2-methylimidazole, 4-methylimidazole, β -myrcene, ochratoxin A, pulegone, α,β -thujone and zeaxenone.

2.2. NOAEL/LOAEL analysis

To investigate the dependency of the toxic potency on exposure duration, we calculated two different ratios of NOAELs or LOAELs from studies with different exposure duration. Firstly, ratios for NOAELs and LOAELs were based on the dose (i.e. mg/kg bw/day), as presented in the authority's evaluation. Secondly, if available, ratios were based on the feed concentration of the compound at the NOAEL (or LOAEL). In cases, in which more than one study was available, the most sensitive endpoint that provided the lowest NOAEL (or LOAEL) was used, an approach well accepted in risk assessment to derive health-based guidance values.

To enhance the consistency of the applied assessment criteria used in both studies of a ratio pair, ratios were preferred if both NOAELs (or both LOAELs) were obtained from the same safety evaluation. However, this was not possible in all cases. In the rare cases, in which more than one informative short-term or more than one long-term study was available, the pair with the lowest dose/dose spacing difference for both of the studies involved was selected. In cases, in which more than one evaluation for a given compound was available, the order of preference

Table 1

Ratios of NOAELs and LOAELs from subchronic and chronic toxicity studies in mice.

Compound	CAS No.	Ratio feed concentration (ppm)		Ratio dose (mg/kg bw/d)	
		NOAEL	LOAEL	NOAEL	LOAEL
2,4-Hexadienal	142-83-6			1,0	1,0
2-Methylimidazole	693-98-1	1.0 ^a	1.0		
4-Methylimidazole	822-36-6	0.7 ^a	1.0		
5-Hydroxymethyl-2-furfural	67-47-0			0.5	0.5
Anthraquinone	84-65-1	2.3 ^a	2.3		
Butylhydroquinone	1948-33-0	2.0	2.0	1.9 ^b	1.9 ^b
Citral	5392-40-5	7.8 ^a	7.8		
Di-(2-ethylhexyl) phthalate	117-81-7	0.3 ^a	0.3		
<i>D</i> -Limonene	5989-27-5			1.0	1.0
Geranyl acetate	105-87-3			6.0 ^a	4.0
Indole-3-carbinole	700-06-1			0.2 ^a	0.2
Isoeugenol	97-54-1			6.0 ^a	4.0
Malonaldehyde	24382-04-5			3.0 ^a	2.1
Melamine	108-78-1	2.7 ^a	2.7		
Pulegone	89-82-7			1.0	1.0
Red 2G	2611-82-7	4.0	8.0	3.4 ^b	3.6 ^b
α,β -Thujone	76231-76-0			1.0	1.0
<i>trans</i> -Cinnamaldehyde	14371-10-9	4.1	3.9	3.1 ^b	3.1 ^b
Urotropin	100-97-0	1.0	1.0	1.0	1.0
Zeaxenone	17924-92-4	1.8 ^a	2.0		
β -Myrcene	123-35-3			1.0	1.0
Number of compounds	21	11	11	14	14

^a At least one surrogate NOAEL was derived.

^b These NOAELs and LOAELs were adjusted by the dose decrement factor of 1.6 (see Material and Methods section).

was: 1) EFSA; 2) JECFA; 3) US NTP. We chose this order, because EFSA and JECFA reports provide points of departure derived by an expert panel, whereas NTP reports do not provide such information.

The data were analysed in two ways.

Firstly, the ratio distributions were analysed descriptively. For the sample of NOAEL (or LOAEL) distributions, the geometric mean (GM) and geometric standard deviation (GSD) of the ratio distributions were determined because the ratio distributions were skewed. The available database was too small to make clear statements about the type of distribution. Therefore, the use of GM, which is not very sensitive for outliers, seemed justified to us, especially in order to be comparable with other similar studies. A GM of the NOAEL (or LOAEL) ratios close to 1 was interpreted as the duration of exposure being of little importance for the NOAEL (or LOAEL). Secondly, the group of short-term and long-term NOAELs (or LOAELs) paired by the investigated substances was examined with the Wilcoxon signed-rank test (IBM SPSS Statistics Version 24, significance level of 0.05) to determine whether the exposure duration has a statistically significant influence on the NOAELs (or LOAELs).

3. Results

3.1. Limitations of the present study

We initially focused our analysis on food additives. In contrast to the pesticide area, data on food additives were in many cases limited or restricted to a single subchronic or chronic study, which was considered appropriate by authorities to derive a health-based guidance value. NOAEL_{sc}/NOAEL_c or LOAEL_{sc}/LOAEL_c study pairs were only identified in trials with rodents (mice or rats), whereas dog studies were scarce. Since approved food additives by definition must be safe, the toxic potency of these compounds is generally very low. Frequently, no adverse effects up to the highest dose tested were observed in repeated dose toxicity studies on food additives. The highest dose was often in the range of grams/kg bw/day, and the administration of even higher doses (i.e. higher than 2 g/kg bw/day) to derive a LOAEL is not practical or

Table 2

Ratios of NOAELs and LOAELs from subchronic and chronic toxicity studies in rats.

Compound	CAS No.	Ratio feed concentration (ppm)		Ratio dose (mg/kg bw/d)	
		NOAEL	LOAEL	NOAEL	LOAEL
2,4-Hexadienal	142-83-6			2.7	2.7
2-Methylimidazole	693-98-1	2.1 ^a	2.1		
4-Methylimidazole	822-36-6	2.0	2.0		
5-Hydroxymethyl-2-furfural	67-47-0			3.0 ^a	2.0
Amaranth	915-67-3			5.3 ^a	25.0
Anthraquinone	84-65-1	4.0 ^a	4.0		
Azorubine	3567-69-9			4.7 ^b	2.9 ^b
Butylhydroquinone	1948-33-0	6.0 ^a	4.0	6.8 ^{a,b}	4.5 ^b
Butylhydroxyanisole	25013-16-5			0.9	1.1
Citral	5392-40-5	0.7 ^a	1.0		
Curcumin	458-37-7	2.5	1.0	1.8 ^b	0.8 ^b
Di-(2-ethylhexyl) phthalate	117-81-7	0.7 ^a	0.4		
D-Limonene	5989-27-5			2.0	2.0
Erythrosine	16423-68-0	0.3	0.3	0.2	0.2
Eugenol	97-53-0	1.0	1.0		
Geranyl acetate	105-87-3			6.0 ^a	4.0
Indole-3-carbinole	700-06-1			0.3 ^a	0.3
Isoeugenol	97-54-1			1.0	1.0
Malonaldehyde	24382-04-5			3.6 ^a	2.5
Melamine	108-78-1	0.3 ^a	0.3		
Ochratoxin A	303-47-9			1.0	1.0
Ponceau 4R	2611-82-7			3.3	0.7
Pulegone	89-82-7			1.5 ^a	1.0
α,β-Thujone	76231-76-0			3.0 ^a	2.0
trans-Cinnamaldehyde	14371-10-9	2.0	2.0	1.9	1.8
Zearalenone	17924-92-4	3.6 ^a	4.0		
β-Myrcene	123-35-3			1a	1
Number of compounds	27	12	12	19	19

^a At least one surrogate NOAEL was derived.

^b These NOAELs and LOAELs were adjusted by the dose decrement factor of 1.6 (see Material and Methods section).

useful. Thus, a wide range of substances could not be included in the present analysis. To overcome the limitations of studies on food additives and to obtain a sufficient number of food-related compounds for our analysis, we subsequently included studies on substances occurring in food conducted by NTP. Previous studies have already used certain NTP studies (Kalberlah and Schneider, 1998, see also Table 4). A major advantage of NTP studies is the availability of both subchronic and chronic studies routinely conducted using the same study design and similar experimental protocol. Thus, in this case, the results of subchronic and chronic studies are very well comparable. Furthermore, most compounds were tested simultaneously in rats and in mice, therefore enabling species comparison. However, chronic NTP studies in some cases not do report haematology and clinical chemistry parameter, which are reported in other chronic studies and this might represent a source of uncertainty. All substances, for which both subchronic and chronic studies in mice and/or rats were identified, are listed in Table 1 (mice) and Table 2 (rats).

3.2. Ratios of NOAELs (or LOAELs) from subchronic and chronic toxicity studies

At least two ratios were calculated for each compound and species: NOAEL_{sc}/NOAEL_c and LOAEL_{sc}/LOAEL_c based on the concentration of the test compound in feed, and/or NOAEL_{sc}/NOAEL_c and LOAEL_{sc}/LOAEL_c based on the administered dose. In studies in which no NOAEL could be experimentally identified, a surrogate NOAEL was calculated by dividing the LOAEL by a factor of 3 (as previously suggested by Braakhuis et al. (2018)). We are aware that this practice increases the uncertainty of our analysis because the real factor for individual compounds might be significantly different. In mice, 21 compounds fulfilled our selection criteria and were analysed (Table 1). In rats, 27 compounds

Fig. 1. Graphical illustration of subchronic to chronic NOAEL and LOAEL ratios in mice and rats (data from Tables 1 and 2).

were analysed (Table 2).

In both species, most of the ratios calculated were between 0.5 and 4.5. Only a few of the calculated ratios were lower (<0.5) or higher (>4.5) (see Fig. 1). These were mostly restricted to studies, in which a surrogate NOAEL had to be used (e.g. NOAEL_c for isoeugenol in mice). However, in general, results obtained by estimation of a surrogate NOAEL showed the same trend as ratios obtained from experimentally obtained NOAELs.

Table 3

Statistical analysis of subchronic and chronic NOAELs and LOAELs based on feed concentrations (mg/kg feed [ppm]) and dose (mg/kg bw/day) in mouse and rat oral toxicity studies.

	Mouse, ratios subchronic vs. chronic				Rat, ratios subchronic vs. chronic			
	NOAEL ^a	LOAEL ^a	NOAEL ^b	LOAEL ^b	NOAEL ^a	LOAEL ^a	NOAEL ^b	LOAEL ^b
GM	1.8	2.0*	1.5 (1.7)	1.4* (1.5)	1.4	1.3	1.9* (2.1)	1.5 (1.7)
GSD	2.5	2.6	2.4 (2.6)	2.2 (2.4)	2.6	2.6	2.6 (2.6)	2.9 (2.9)
Ratio ≤ 1 (%)	36	36	50 (50)	57 (57)	43	50	32 (32)	42 (37)

GM, geometric mean; GSD, geometric standard deviation; rounded up to 1 digit. The numbers given in parentheses were not adjusted for dose decrement observed in feeding studies.

*Short-term and long-term NOAELs, or LOAELs, in this group statistically significantly differ in the Wilcoxon signed-rank test ($p < 0.05$).

^a Ratio of NOAELs/LOAELs based on compound concentration in feed (ppm).

^b Ratio of NOAELs/LOAELs based on the dose (mg/kg bw/day).

4. Discussion

Geometric means (GM) and geometric standard deviations (GSD) of short-term and long-term NOAEL (and LOAEL) ratios and a statistical analysis of the underlying NOAELs (and LOAELs) are summarized in Table 3. The GM values ranged from 1.0 to 2.0, the GSD values from 2.2 to 4.2 and in a few cases the difference between short-term and long-term NOAELs (and LOAELs) reached statistical significance. These findings very well match with results reported in previous studies (see Table 4). In case that a short-term study NOAEL is equal to a chronic NOAEL, the NOAEL ratio is 1, and less than 1 if the short-term study NOAEL is lower. Ratios less than or equal to 1 were observed for 32–57% of the compounds (Table 3), thus indicating that in many cases short-term NOAELs are lower than long-term NOAELs, a finding previously reported for pesticides by Zarn et al. (2011). This questions the hypothesis that a prolonged exposure exacerbates the toxic potency, which would be indicated by decreasing NOAELs. However, it should be noted that the total number of ratios, which could be used for a statistical analysis in this study, was rather low and, therefore, the analysis has less statistical power when compared to previous analyses performed with pesticides (Zarn et al., 2011; Zarn et al., 2013; Zarn and O'Brien, 2018).

The numbers given in parentheses are the values calculated based on NOAEL and LOAEL values not adjusted for the dose decrement observed in rodent feeding studies (see Material and Methods section). No statistical analysis was performed with these values.

The compound with the highest LOAEL ratio of 25 in rats was the food colouring agent amaranth. This high ratio could be explained by a different interpretation of the results of the chronic toxicity study used for the evaluation. JECFA assessed this study (Clode et al., 1987) and identified the NOAEL at 50 mg/kg bw/d and the LOAEL at 250 mg/kg bw/day based on renal pelvic calcification and renal pelvic epithelial hyperplasia. In contrast, EFSA concluded that a definitive NOAEL could not be identified in this study and 50 mg/kg bw/day should be considered as a LOAEL. Applying an uncertainty factor of 3, a NOAEL of 15 mg/kg bw/day was calculated by EFSA. In the subchronic study a NOAEL of 80 mg/kg bw/day and a LOAEL of 1250 mg/kg bw/day based on an increase in the number of animals with renal pelvic hyperplasia and calcification were identified. This study was not re-evaluated by EFSA on the basis of stricter criteria, so that both studies are difficult to compare. In addition, the dose spacing (DS) in the subchronic study is rather high (DS = 16x) if compared to the chronic study (DS = 5x). Overall, this led to the high LOAEL ratio.

Besides dose spacing, differences in animal group size providing substantially different statistical power of subchronic vs. chronic studies may also contribute to the variance in ratios. Zarn et al. (2011) reported that ratios decreased the higher the animal number in short-term studies and the lower the dose-spacing in long-term studies are (Zarn et al., 2011). Furthermore, usually more detailed analyses are performed in chronic studies as compared to short-term studies (Zarn and O'Brien, 2018). Due to the limited data set, a statistical analysis for dose spacing and animal numbers as covariates could not be performed in the present

study.

4.1. Comparison with literature data

An increasing number of studies investigated the impact of study duration on the NOAEL or LOAEL (see Table 4; also summarized by Zarn et al. (2011) and Zarn and O'Brien (2018)) by comparing acute with subacute, subacute with subchronic, subacute with chronic and subchronic with chronic NOAELs and/or LOAELs. The great majority of these evaluations were based on studies on pesticides or industrial chemicals, for which large databases are available; some of them also analysed studies with pharmaceuticals.

In these studies, the GM (GSD) of ratios was reported as follows: ratios subacute to subchronic: 1.3–3.3 (2.1–3.8); ratios subacute to chronic: 3.2–5.1 (3.6–4.7) and ratios subchronic to chronic: 1.2–4.35 (2.1–3.9). Overall, the GMs of the NOAEL ratios were quite similar in all these studies, i.e. in the range of 2–3 (1.3–5.1), whereas the GSD ranged from 2.1 to 4.7. This variation might be due to the use of different species, variable size of the database, and diverse criteria for data selection (Vermeire et al., 1999). A frequent conclusion from these studies is that short-term studies give rise to higher NOAELs than long-term studies. However, it has been shown that certain study design factors can have a considerable influence on NOAEL or LOAEL distributions, such as the number of animals per group, dose spacing and dose decrement in feeding studies (Batke et al., 2011; Escher et al., 2020; Zarn et al., 2011; Zarn et al., 2013; Zarn and O'Brien, 2018). If the NOAELs are corrected for these study design factors, they do not significantly differ from each other, and comparable potencies after short-term and chronic exposures are obtained (Zarn et al. 2011, 2013).

The NOAEL-based approach has been criticized, since NOAELs are crucially dependent on dose selection. Thus, differences in dose spacing between short- and long-term studies in a study pair influence the NOAEL ratios (Batke et al., 2011; Lampe et al., 2018). As an alternative, the use of Benchmark Dose Modelling (BMD) to derive ratios for study pairs has been suggested, which eliminates the influence of dose spacing (Lampe et al., 2018). For example, Bokkers and Slob (2005) compared ratio distributions based on the NOAEL and the BMD approach for subchronic and chronic study pairs (see Table 4). The GM values of the NOAEL and BMD ratio distributions were similar; but the GSDs differed considerably (Bokkers and Slob, 2005). The authors concluded that application of the BMD approach results in less wide distributions (Bokkers and Slob, 2005). Furthermore, by applying the BMD approach, a larger fraction of the available datasets, i.e. also studies providing no NOAEL and/or LOAEL, could be used to derive a ratio (Bokkers and Slob, 2005). NOAEL ratios could be derived in only 68 of 314 dose-response datasets on body weights and liver weights of mice and rats, while BMD ratios could be derived in 189 datasets (Bokkers and Slob, 2005). In another study, Lampe et al. (2018) analysed ratio distributions of NOAELs and BMDs derived from subacute (28-day) and subchronic (90-day) studies. The distribution of the BMD ratios was not significantly different from the distribution of NOAEL ratios, but the BMD ratio

Table 4

Overview of studies investigating the influence of exposure duration on points of departure.

Compound class	Source of dataset	Application/ Species	Number of compounds	Number of ratios	Duration	Outcome (as reported by the authors)	NOAEL/ LOAEL ratio, GM (GSD)	Reference
Acute vs. subacute/subchronic								
New chemicals notified in Europe from 1981	New Chemical Database/EU Joint Research Centre (JRC, Ispra)	Oral	1552		Acute (LD ₅₀) vs. 28 d (NOAEL)	NOAEL values ≥ 200 mg/kg obtained from 28 days: This threshold is suitable to correctly identify 63% of the non-toxic substances (LD ₅₀ > 2000 mg/kg).		Bulgheroni et al. (2009)
	Agency for Toxic Substances and Disease Registry (ATSDR)	Oral	123		Acute (up to 14 d) vs. intermediate (15–364 d) (LOAEL)	Impact of chemical structure and chemical structure activity (chemicals grouped together): selected endpoints can influence the prediction of extrapolations across durations of exposure.	25.6 (average ratio)	Pohl et al. (2010)
	ECHA/REACH, IUCLID database	Oral	1256		Acute (LD ₅₀) vs. 28 d (NOAEL)	415 substances showed low toxicity in the sub-acute toxicity study (i.e., NO(A)EL ≥ 1000 mg/kg). For 98% of these substances, low acute oral toxicity was also reported (i.e., LD ₅₀ above the classification threshold of 2000 mg/ kg).		Gissi et al. (2017)
Subacute vs. subchronic								
Pesticides, stabilizers/ plasticizers, food additives, disinfectants	TNO pesticide dossiers, Dutch National Institute of Public Health (DNIPH)	Oral, rat		82	Subacute (14 d) vs. subchronic	For 56% of the compounds reviewed, the NOAEL subacute was equal to the NOAEL subchronic. In about 25% of the compounds reviewed, the NOAEL subchronic was lower than the NOAEL subacute.	2 (95th perc. 6.6)	(Woutersen et al., 1984) as interpreted by Kalberlah et al. (2002)
	Dutch National Institute of Public Health (DNIPH)	Inhalation, various species		91	Subacute (14 d) vs. subchronic		2.2 (95th perc. 62.0)	(Kramer et al., 1995) as interpreted by Kalberlah et al. (2002)
Heterogenous	NTP studies	Oral (gavage), rats and mice		a) 87 (rats) b) 78 (mice)	Subacute vs. subchronic		a) 3.3 (95th perc. 10.0) b) 2.6 (95th perc. 10.0)	(Kalberlah and Schneider, 1998) as interpreted by Kalberlah et al. (2002)
Chemicals	Industry data	Oral, rats		21	Subacute vs. subchronic		2.1 (95th perc. 8.1)	(Kalberlah and Schneider, 1998) as interpreted by Kalberlah et al. (2002)
Pesticides and other chemicals	TNO, IPCS and JMPR	Oral, rats and mice	198	a) 25 (rats and mice) b) 22 (rats)	Subacute (21–42 d) vs. subchronic (49–183 d)		a) 1.6 (3.3) (95th perc. 11.4) b) 1.6 (3.5) (95th perc. 13.0)	Groeneveld et al. (2004)

(continued on next page)

Table 4 (continued)

Compound class	Source of dataset	Application/ Species	Number of compounds	Number of ratios	Duration	Outcome (as reported by the authors)	NOAEL/ LOAEL ratio, GM (GSD)	Reference
Industrial chemicals	RepDose, www.fr-aunhofer-repdose.de (Bitsch et al., 2006)	Oral, inhalation, rodents		38	20–33 d vs. 83–99 d		1.3 (2.4)	Batke et al. (2011)
Pesticides	Evaluations of pesticides of EFSA, JMPR and U.S. EPA	Oral, rats and mice		a)100 (rats) b) 28 (mice)	Subacute vs. subchronic		a) 1.9 (3.0) b) 1.6 (2.1)	Zarn et al. (2011)
Industrial chemicals	ECHA CHEM, eChemPortal	Oral, rats	21		28 d vs. 90 d	Analysis of 28- and 90-day studies of industrial chemicals with low toxicity lead to a set of criteria for predicting a 'low (sub) acute toxicity profile' without requiring a 90-day study.		(Taylor and Andrew, 2017; Taylor et al., 2014)
Environmental chemicals	Japan Pollutant and Transfer Register	Oral, rats	167		Subacute vs. subchronic	NOEL ratios are not dependent on endpoint/target organ (liver, kidney, blood, body weight). Assessment factors related to exposure duration leading to effects involving liver, kidney and body weight need not be treated independently with exception of effects on blood.		Takeshita et al. (2014)
Candidate drugs	AstraZeneca		39		≤6 weeks vs. ≥13 weeks	Target organ toxicities of candidate drugs show partial or complete recovery or progress in severity, despite continued exposure to the drug.		Roberts et al. (2015)
Industrial chemicals	ECHA CHEM, eChemPortal		305		28 d vs. 90 d	Lack of toxicity in a 28-day study is a relatively good predictor that a chemical will be non-toxic acutely, but weaker support for the hypothesis that a 90-day dose can be extrapolated from a 28-day dose.		Luechtefeld et al. (2016)
Industrial and environmental chemicals	ECHA CHEM, ATSDR, NSF risk assessments	Oral, rats and mice		a)104 b)34 (similar dose spacing and quality)	28 d (21–33 d) vs. 90 d (83–99 d)	A default 10-fold extrapolation factor in chemical risk assessment applications is sufficient to account for the uncertainty associated with evaluating human health risk based on results from a 28-day study in the absence of results from a 90-day study.	a) 1.3 (3.8) (95th perc. 10.0) b) 1.5 (2.8) (95th perc. 8.4)	Lampe et al. (2018)
Industrial chemicals, pesticides, pharmaceuticals	ECHA CHEM, ToxRef, Hess DB, IMI eTOX, ELINCS, RepDose	a) Oral, rats and mice b) Inhalation, rats and mice	a) 172 b) 44	a) 302 b) 67	28 d (20–35 d) vs. 90 d (82–121 d)	The impact of a longer treatment period on the study N(L)OEL is on average not high. Exclusion of confounding factors such as deviation in dose selection and spacing resulted in GM values of 1.5.	a) 1.6 (4.1) b) 1.7 (4.8)	Escher et al. (2020)

(continued on next page)

Table 4 (continued)

Compound class	Source of dataset	Application/ Species	Number of compounds	Number of ratios	Duration	Outcome (as reported by the authors)	NOAEL/ LOAEL ratio, GM (GSD)	Reference
Subacute vs. chronic								
Heterogenous	RIVM's Advisory Centre of Toxicology; IPCS, ATSDR			71	Subacute vs. chronic		4.1 (4.4)	Kramer et al. (1996)
Heterogenous	NTP studies	Oral (gavage), rats and mice		a) 76 (rats) b) 51 (mice)	Subacute vs chronic		a) 5.1 (95th perc. 14.1) b) 4.2 (95th perc. 10.6)	(Kalberlah and Schneider, 1998) as interpreted by Kalberlah et al. (2002)
Chemicals	Industry data	Oral, rats		21	Subacute vs chronic		3.2 (95th perc. 14.7)	(Kalberlah and Schneider, 1998) as interpreted by Kalberlah et al. (2002)
Pesticides and other chemicals	TNO, IPCS and JMPR	Oral, rats and mice	198	a) 35 (rats and mice) b) 25 (rats)	Subacute (21–42 d) vs. chronic (≥365 d)		a) 4.9 (3.5) b) 5.8 (3.5)	Groeneveld et al. (2004)
Industrial chemicals	RepDose, www.fr.aunhofer-repdose.de (Bitsch et al., 2006)	Oral, rodents		14	20–33 d vs. >699 d	NOAELs reduce over time by as much as 3.4 fold between subacute and chronic studies.	3.4 (3.7)	Batke et al. (2011)
Pesticides	Evaluations of pesticides of EFSA, JMPR and U.S. EPA	Oral, rats and mice		a) 107 (rats) b) 56 (mice)	Subacute vs. chronic		a) 4.3 (4.7) b) 3.4 (3.6)	Zarn et al. (2011)
Pesticides	Evaluations of pesticides of EFSA, JMPR and U.S. EPA	Oral, rats		129	Subacute vs. chronic	NOAELs of pesticides remain statistically unchanged in developmental, subacute, subchronic, reproductive and chronic toxicity studies covering exposure durations between 2 and 104 weeks.	2.1	Zarn and O'Brien (2018)
Subchronic vs. chronic								
		Oral, various species	33		Subchronic vs. chronic		2.2 (90th perc. 5.8)	(Weil and McCollister, 1963) as interpreted by Kalberlah et al. (2002)
Chemicals	Industry data	Oral, rats and mice		a) 24 (rats) b) 18 (mice)	Subchronic vs. chronic		a) 1.7 (95th perc. 5.0) b) 2.0 (95th perc. 5.0)	(Kalberlah and Schneider, 1998) as interpreted by Kalberlah et al. (2002)
Heterogenous	NTP studies	Oral (gavage), rats and mice		a) 71 (rats) b) 55 (mice)	Subchronic vs. chronic		a) 2.9 (95th perc. 8.6) b) 2.5 (95th perc 6.0)	(Kalberlah and Schneider, 1998) as interpreted by Kalberlah et al. (2002)
Pesticides (50%), solvents (25%), metal containing compounds, phthalates, others	TOXbank (RIVM), evaluations by IPCS, ATSDR	a) Oral, rats and mice combined b) Oral, rats		a) 149 b) 70	subchronic vs. chronic		a) 1.7 (5.6) b) 1.5 (6.3)	Pieters et al. (1998)
Pesticides	Confidential reports submitted by pesticide	Oral, dogs	172	141	Subchronic vs. chronic	There was a significant correlation between the LOELs determined in subchronic studies		Spielmann and Gerbracht (2001)

(continued on next page)

Table 4 (continued)

Compound class	Source of dataset	Application/ Species	Number of compounds	Number of ratios	Duration	Outcome (as reported by the authors)	NOAEL/ LOAEL ratio, GM (GSD)	Reference
	manufacturers to German BgVV					and those determined in chronic studies in dogs ($r = 0.78-0.84$), but a significant difference between subacute studies and subchronic or chronic studies.		
Pesticides and other chemicals	TNO, IPCS and JMPR	Oral, rats and mice	198	a) 70 (rats and mice) b) 56 (rats)	Subchronic (49–183 d) vs. chronic (≥ 365 d)		a) 2.25 (3.6) b) 2.28 (3.8)	Groeneweld et al. (2004)
Heterogenous	NTP	Oral	31	a) 53 (NOAEL) b) 53 (CED, critical effect dose)	Subchronic vs. chronic		a) 1.2 (3.3) b) 1.6 (2.3)	Bokkers and Slob (2005)
	ATSDR	Oral	81		Intermediate (15–364 d) vs. chronic (>365 d) (LOAELs)		4.35 (average ratio)	Pohl et al. (2010)
Pesticides	Evaluations of pesticides of EFSA, JMPR and U.S. EPA	Oral, rats and mice		a) 222 (rats) b) 99 (mice)	Subchronic vs. chronic		a) 2.5 (3.4) b) 2.2 (3.9)	Zarn et al. (2011)
Industrial chemicals	RepDose, www.fr.aunhofer-repdose.de (Bitsch et al., 2006)	Oral, rodents		58	83–99 d vs. >699 d		1.4 (2.1)	Batke et al. (2011)
Pesticides	Evaluations of pesticides of EFSA, JMPR and U.S. EPA	Oral, rats		253	Subchronic vs. chronic		1.7	Zarn and O'Brien (2018)
Non-genotoxic carcinogens: chemicals and pharma- ceuticals	ECHA, EFSA, WHO, IARC, ATSDR websites. Pharmaceuticals: Medicines Evaluation Board (MEB) of the Netherlands		44 (34 chemicals, 10 pharmaceuticals)		Subchronic vs. chronic (carcinogenicity study)	NOAELs from the subchronic studies show a good correlation with the NOAELs (and BMDs) from the carcinogenicity studies.		Braakhuis et al. (2018)
Industrial chemicals, pesticides, pharma- ceuticals	ECHA CHEM, ToxRef, Hess DB, IMI eTOX, ELINCS, RepDose	a) Oral, rats and mice b) Inhalation, rats and mice	a) 462 b) 71	a) 1059 b) 226	90 days (82–121 days) vs. ≥ 350 days	The impact of a longer treatment period on the study N(LO)EL is on average not high. Exclusion of confounding factors such as deviation in dose selection and spacing resulted in GM values of 1.5.	a) 1.8 (5.0) b) 1.5 (3.8)	Escher et al. (2020)

ATSDR, Agency for Toxic Substances and Disease Registry; d, days; ECHA, European Chemicals Agency; IARC, International Agency for Research on Cancer; IPCS, International Programme on Chemical Safety; JMPR, Joint FAO/WHO Meeting on Pesticide Residues; NTP, National Toxicology Program; perc., percentile; TNO, the Netherlands Organisation for applied scientific research; US-EPA, U.S. Environmental Protection Agency; WHO, World Health Organization.

distribution had a lower GM and a lower 95th percentile (Lampe et al., 2018). However, compared to Bokkers and Slob (2005), Lampe et al. used a smaller dataset (31 toxicological endpoints among 16 study pairs from 15 chemicals) and thus, the results are based on a dataset with a lower statistical power. In the present study, the BMD approach was not applied due to the lack of dose-response data for all endpoints in many studies.

In a recent study, it has been demonstrated that even in the case of non-genotoxic carcinogens NOAELs from short-term studies are comparable to those from long-term studies (Braakhuis et al., 2018). In this analysis of BMDs calculated from carcinogenicity data of 44 non-genotoxic carcinogens (chemicals and pharmaceuticals), NOAELs and BMD values derived from the carcinogenicity studies showed a good correlation with NOAELs derived from subchronic toxicity studies. The

authors concluded that in case that a subchronic toxicity study is available a carcinogenicity study provides little added value for the derivation of a health-based guidance value for a possibly carcinogenic non-genotoxic compound, e.g. under REACH (Registration, Evaluation, Authorization and Restriction of Chemicals) legislation (Braakhuis et al., 2018).

For pharmaceuticals, similar observations have been made (Horner et al. 2013, 2014; Roberts et al., 2015). An analysis of 39 pharmaceutical compounds assessed the progression of target organ toxicities from the one month first-time-in-man (FTiM) studies to longer-term studies conducted at comparable doses to test the hypothesis that toxicity (in terms of severity) is exacerbated by prolonged exposure (Roberts et al., 2015). The results of their analysis challenged this hypothesis by demonstrating that target organs more likely show partial or complete

recovery rather than progression, despite continued exposure to the drug (Roberts et al., 2015).

5. Conclusion

Although the present analysis on food additives, natural food constituents and contaminants was based on a relatively small data set due to the limited availability of study pairs, our results are in accordance with literature data on pesticides, pharmaceuticals and industrial chemicals and may have important implications for the current and future safety assessment of food/food constituents. In view of the legal requirement to replace, reduce and refine (3R) the use of laboratory animals wherever possible (see Directive 2010/63/EU as amended by Regulation (EU) 2019/1010), the finding that subchronic toxicity studies can lead to comparable health-based guidance values as chronic studies questions the added value of chronic studies for the derivation of reference points such as NOAELs and BMDs. Particularly for compounds not intentionally added to food, chronic toxicity studies are not always available and, thus, risk assessment may have to rely on results of short-term studies. In the present study, the GM of ratios of subchronic to chronic NOAELs and LOAELs ranged between 1 and 2. Some substances showed higher ratios, such as citral (ratio 7.8 in mice) or butylhydroquinone (ratio 6.0 in rats), thereby leading to an overall GM ranging up to 2.0 (Table 3). Our study results very well agree with previously published data, which reported quite similar GM ratios in the range of 1–4. The observed variation of GM values might be due to the use of different species, variable size of database, and diverse criteria for data selection. Thus, our data support the application of an additional uncertainty factor of 2 for the extrapolation from a subchronic toxicity study to chronic toxicity as recommended by EFSA, provided the subchronic toxicity study is of adequate quality (EFSA, 2012b).

Taken together, our data support results of previous analyses, which suggest that well conducted and high-powered subchronic studies may be useful in identifying a point of departure for risk assessment.

Compliance with ethical standards

This article does not contain any studies with human participants or animals performed by any of the authors.

Funding

This work was primarily supported by the German Research Foundation (Deutsche Forschungsgemeinschaft, DFG), HE 2509/15-11. Further support was provided by the DK-EPA (MST-667-00205) and the European Union's Horizon 2020 Research and Innovation Programme under grant agreements No. 681002 (EU-ToxRisk). The funding sources did not have an influence on the collection, analysis and interpretation of the data, in the writing of the report and in the decision to submit the article for publication.

Credit author statement

Sabine Guth: Investigation (data collection, data analysis and interpretation), Writing - Original Draft Preparation, Writing - Review & Editing. **Angelika Roth:** Investigation (data collection, data analysis and interpretation), Writing - Original Draft Preparation. **Barbara Engeli:** Investigation (data analysis and interpretation), Writing - Review & Editing. **Dirk W. Lachenmeier:** Validation, Writing - Review & Editing. **Alexander T. Cartus:** Validation, Writing - Review & Editing. **Stephanie Hüser:** Investigation (data analysis and interpretation), Writing - Review & Editing. **Matthias Baum:** Writing - Review & Editing. **Patrick Diel:** Writing - Review & Editing. **Gerhard Eisenbrand:** Writing - Review & Editing. **Jan G. Hengstler:** Writing - Review & Editing, Supervision. **Hans Ulrich Humpf:** Writing - Review & Editing. **Hans-Georg Joost:** Writing - Review & Editing. **Alfonso Lampen:**

Writing - Review & Editing. **Marcel Leist:** Writing - Review & Editing. **Doris Marko:** Writing - Review & Editing. **Pablo Steinberg:** Conceptualization, Writing - Review & Editing. **Angela Mally:** Conceptualization, Writing - Review & Editing, Supervision. **Jürg Zarn:** Conceptualization, Methodology, Formal analysis, Writing - Review & Editing, Visualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This study was an activity of the working group on food constituents of the Senate Commission on Food Safety (SKLM) of the German Research Foundation (DFG). The authors wish to thank the members of the SKLM for fruitful discussions and the DFG for their continuous support of the SKLM Commission.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.fct.2020.111784>.

References

- Batke, M., Escher, S., Hoffmann-Doerr, S., Melber, C., Messinger, H., Mangelsdorf, I., 2011. Evaluation of time extrapolation factors based on the database RepDose. *Toxicol. Lett.* 205 (2), 122–129. <https://doi.org/10.1016/j.toxlet.2011.05.1030>.
- Bitsch, A., Jacobi, S., Melber, C., Wahnschaffe, U., Simetska, N., Mangelsdorf, I., 2006. REPDOSE: a database on repeated dose toxicity studies of commercial chemicals—A multifunctional tool. *Regul. Toxicol. Pharmacol.* 46 (3), 202–210. <https://doi.org/10.1016/j.yrtph.2006.05.013>.
- Bokkers, B.G., Slob, W., 2005. A comparison of ratio distributions based on the NOAEL and the benchmark approach for subchronic-to-chronic extrapolation. *Toxicol. Sci.* 85 (2), 1033–1040. <https://doi.org/10.1093/toxsci/kfi144>.
- Box, R.J., Spielmann, H., 2005. Use of the dog as non-rodent test species in the safety testing schedule associated with the registration of crop and plant protection products (pesticides): present status. *Arch. Toxicol.* 79 (11), 615–626. <https://doi.org/10.1007/s00204-005-0678-0>.
- Braakhuis, H.M., Slob, W., Olthof, E.D., et al., 2018. Is current risk assessment of non-genotoxic carcinogens protective? *Crit. Rev. Toxicol.* 48 (6), 500–511. <https://doi.org/10.1080/10408444.2018.1458818>.
- Bulgheroni, A., Kinsner-Ovaskainen, A., Hoffmann, S., Hartung, T., Prieto, P., 2009. Estimation of acute oral toxicity using the No Observed Adverse Effect Level (NOAEL) from the 28 day repeated dose toxicity studies in rats. *Regul. Toxicol. Pharmacol.* 53 (1), 16–19. <https://doi.org/10.1016/j.yrtph.2008.10.001>.
- Clode, S.A., Hooson, J., Grant, D., Butler, W.H., 1987. Long-term toxicity study of amaranth in rats using animals exposed in utero. *Food Chem. Toxicol.* 25 (12), 937–946. [https://doi.org/10.1016/0278-6915\(87\)90287-0](https://doi.org/10.1016/0278-6915(87)90287-0).
- Dellarco, V.L., Rowland, J., May, B., 2010. A retrospective analysis of toxicity studies in dogs and impact on the chronic reference dose for conventional pesticide chemicals. *Crit. Rev. Toxicol.* 40 (1), 16–23. <https://doi.org/10.3109/10408440903401529>.
- Doe, J.E., Boobis, A.R., Blacker, A., et al., 2006. A tiered approach to systemic toxicity testing for agricultural chemical safety assessment. *Crit. Rev. Toxicol.* 36 (1), 37–68.
- ECHA, 2012. *Guidance on Information Requirements and Chemical Safety Assessment Chapter R.8: Characterisation of Dose [concentration]-Response for Human Health*. ECHA, Helsinki, Finland.
- EFSA, 2012a. Panel on food additives and nutrient sources added to food (ANS); guidance for submission for food additive evaluations. *EFSA J.* 10 (7), 2760. <https://doi.org/10.2903/j.efsa.2012.2760> [60 pp.].
- EFSA, 2012b. Scientific Committee; Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data. *EFSA J.* 10 (3), 2579. <https://doi.org/10.2903/j.efsa.2012.2579>.
- Escher, S.E., Mangelsdorf, I., Hoffmann-Doerr, S., et al., 2020. Time extrapolation in regulatory risk assessment: the impact of study differences on the extrapolation factors. *Regul. Toxicol. Pharmacol.* 112, 104584. <https://doi.org/10.1016/j.yrtph.2020.104584>.
- Gissi, A., Louekari, K., Hoffstadt, L., Bornatowicz, N., Aparicio, A.M., 2017. Alternative acute oral toxicity assessment under REACH based on sub-acute toxicity values. *ALTEX* 34 (3), 353–361. <https://doi.org/10.14573/altex.1609121>.
- Groeneveld, C.N., Hakker, B.C., Bos, P.M.J., Heer, C., 2004. Extrapolation for exposure duration in oral toxicity: a quantitative analysis of historical toxicity data. *Hum. Ecol. Risk Assess.* 10, 709–716.

- Horner, S., Robinson, S., Lees, D., Callander, R., Roberts, R., 2014. Target organ profiles in toxicity studies supporting human dosing: an assessment of recovery and chronic dosing. *Regul. Toxicol. Pharmacol.* 70 (1), 270–285. <https://doi.org/10.1016/j.yrtph.2014.07.008>.
- Horner, S., Ryan, D., Robinson, S., Callander, R., Stamp, K., Roberts, R.A., 2013. Target organ toxicities in studies conducted to support first time in man dosing: an analysis across species and therapy areas. *Regul. Toxicol. Pharmacol.* 65 (3), 334–343. <https://doi.org/10.1016/j.yrtph.2013.02.002>.
- Janer, G., Hakkert, B.C., Piersma, A.H., Vermeire, T., Slob, W., 2007a. A retrospective analysis of the added value of the rat two-generation reproductive toxicity study versus the rat subchronic toxicity study. *Reprod. Toxicol.* 24 (1), 103–113. <https://doi.org/10.1016/j.reprotox.2007.05.004>.
- Janer, G., Hakkert, B.C., Slob, W., Vermeire, T., Piersma, A.H., 2007b. A retrospective analysis of the two-generation study: what is the added value of the second generation? *Reprod. Toxicol.* 24 (1), 97–102. <https://doi.org/10.1016/j.reprotox.2007.04.068>.
- Kalberlah, F., Fost, U., Schneider, K., 2002. Time extrapolation and interspecies extrapolation for locally acting substances in case of limited toxicological data. *Ann. Occup. Hyg.* 46 (2), 175–185. <https://doi.org/10.1093/annhyg/mef014>.
- Kalberlah, F., Schneider, K., 1998. Quantification of Extrapolation Factors. Final Report of Research Project No. 116 06 113 of the Federal Environmental Agency. Federal Institute for Occupational Safety and Health, Dortmund, Germany.
- Kobel, W., Fegert, I., Billington, R., et al., 2010. A 1-year toxicity study in dogs is no longer a scientifically justifiable core data requirement for the safety assessment of pesticides. *Crit. Rev. Toxicol.* 40 (1), 1–15. <https://doi.org/10.3109/10408440903300098>.
- Kramer, H.J., van den Ham, W.A., Slob, W., Pieters, M.N., 1995. Derivation of Conversion Factors to Estimate an Indicative Chronic NOAEL from Short-Term Toxicity Data. National Institute of Public Health and the Environment, Bilthoven.
- Kramer, H.J., van den Ham, W.A., Slob, W., Pieters, M.N., 1996. Conversion factors estimating indicative chronic no-observed-adverse-effect levels from short-term toxicity data. *Regul. Toxicol. Pharmacol.* 23 (3), 249–255. <https://doi.org/10.1006/rtp.1996.0050>.
- Lampe, B.J., Fuller, E., Kuppusamy, S.P., 2018. A quantitative comparison of points of departure between 28-day and 90-day repeated dose studies with a proposed extrapolation factor. *Regul. Toxicol. Pharmacol.* 92, 189–200. <https://doi.org/10.1016/j.yrtph.2017.12.007>.
- Luechtefeld, T., Maertens, A., Russo, D.P., Rovida, C., Zhu, H., Hartung, T., 2016. Analysis of public oral toxicity data from REACH registrations 2008–2014. *ALTEX* 33 (2), 111–122. <https://doi.org/10.14573/altex.1510054>.
- Pieters, M.N., Kramer, H.J., Slob, W., 1998. Evaluation of the uncertainty factor for subchronic-to-chronic extrapolation: statistical analysis of toxicity data. *Regul. Toxicol. Pharmacol.* 27 (2), 108–111. <https://doi.org/10.1006/rtp.1997.1196>.
- Pohl, H.R., Chou, C.H., Ruiz, P., Holler, J.S., 2010. Chemical risk assessment and uncertainty associated with extrapolation across exposure duration. *Regul. Toxicol. Pharmacol.* 57 (1), 18–23. <https://doi.org/10.1016/j.yrtph.2009.11.007>.
- Roberts, R., Callander, R., Duffy, P., Jacobsen, M., Knight, R., Boobis, A., 2015. Target organ profiles in toxicity studies supporting human dosing: does severity progress with longer duration of exposure? *Regul. Toxicol. Pharmacol.* 73 (3), 737–746. <https://doi.org/10.1016/j.yrtph.2015.10.021>.
- Spielmann, H., Gerbracht, U., 2001. The use of dogs as second species in regulatory testing of pesticides. Part II: subacute, subchronic and chronic studies in the dog. *Arch. Toxicol.* 75 (1), 1–21. <https://doi.org/10.1007/s002040000195>.
- Takeshita, J., Kishimoto, A., Gamo, M., 2014. Investigation of organ-specific assessment factors related to sub-acute and sub-chronic toxicity studies. *Fund Toxicol. Sci.* 1 (1), 1–6.
- Taylor, K., Andrew, D.J., 2017. The added value of the 90-day repeated dose oral toxicity test for industrial chemicals with a low (sub)acute toxicity profile in a high quality dataset: an update. *Regul. Toxicol. Pharmacol.* 90, 258–261. <https://doi.org/10.1016/j.yrtph.2017.09.018>.
- Taylor, K., Andrew, D.J., Rego, L., 2014. The added value of the 90-day repeated dose oral toxicity test for industrial chemicals with a low (sub)acute toxicity profile in a high quality dataset. *Regul. Toxicol. Pharmacol.* 69 (3), 320–332. <https://doi.org/10.1016/j.yrtph.2014.04.008>.
- Vermeire, T., Stevenson, H., Peiters, M.N., Rennen, M., Slob, W., Hakkert, B.C., 1999. Assessment factors for human health risk assessment: a discussion paper. *Crit. Rev. Toxicol.* 29 (5), 439–490. <https://doi.org/10.1080/10408449991349249>.
- Weil, C.S., McCollister, D.D., 1963. Relationship between short- and long-term feeding studies in designing an effective toxicity test. *Agric. Food Chem.* 11, 486.
- Woutersen, R.A., Til, H.P., Feron, V.J., 1984. Sub-acute versus sub-chronic oral toxicity study in rats: comparative study of 82 compounds. *J. Appl. Toxicol.* 4 (5), 277–280. <https://doi.org/10.1002/jat.2550040515>.
- Zarn, J.A., Engeli, B.E., Schlatter, J.R., 2011. Study parameters influencing NOAEL and LOAEL in toxicity feeding studies for pesticides: exposure duration versus dose decrement, dose spacing, group size and chemical class. *Regul. Toxicol. Pharmacol.* 61 (2), 243–250. <https://doi.org/10.1016/j.yrtph.2011.08.004>.
- Zarn, J.A., Engeli, B.E., Schlatter, J.R., 2013. Characterization of the dose decrement in regulatory rat pesticide toxicity feeding studies. *Regul. Toxicol. Pharmacol.* 67 (2), 215–220. <https://doi.org/10.1016/j.yrtph.2013.07.012>.
- Zarn, J.A., Hanggi, E., Kuchen, A., Schlatter, J.R., 2010. The significance of the subchronic toxicity in the dietary risk assessment of pesticides. *Regul. Toxicol. Pharmacol.* 58 (1), 72–78. <https://doi.org/10.1016/j.yrtph.2010.04.007>.
- Zarn, J.A., O'Brien, C.D., 2018. Current pesticide dietary risk assessment in light of comparable animal study NOAELs after chronic and short-termed exposure durations. *Arch. Toxicol.* 92 (1), 157–167. <https://doi.org/10.1007/s00204-017-2052-4>.