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

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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.

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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_/LOAEL_) are lower than NOAELs/LOAELs derived from short-term (subacute, subchronic) toxicity studies (NOAELsa/LOAELsa, NOAELsc/LOAELsc). 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 studies (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).

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, 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

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2 From now on, the subacute, subchronic or chronic toxicity studies will be simply named subacute, subchronic or chronic studies.
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 (Brakhuisk 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, 2016). 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-hydroxyethyl-2-furfural, indole-3-carbonic acid, isoegenol, α-limonene, malonaldehyde, melamine, 2-methylimidazole, 4-methylimidazoozole, β-myrcene, neotame, ochratoxin A, Ponceau 4R, pulegone, Red 2G, sucralose, α,β-thujone, urotropin and zearalenone. 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 (butylhydroxyanisole, 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 contamination, were tested by NTP: anthraquinone, butylhydroquinone, butylhydroxyanisole, α-limonene were identified) were excluded from the analysis, since any assumption of the studies involved was selected. In cases, in which more than one evaluation for a given compound was available, the order of preference 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. NOAELs/NOAEL, or LOAELs/LOAEL 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 NOAEL is not practical or

| 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    | 1.0   |        |
| 4-Methylimidazole | 822-36-6 | 0.7    | 1.0   |        |
| 5-Hydroxymethyl-2-furfural | 67-47-0 | 0.5    | 0.5   |        |
| Anthraquinone | 84-65-1 | 2.3    | 2.3   |        |
| Butylhydroquinone | 1948-33-0 | 2.0    | 2.0   | 1.9    | 1.9    |
| Citral | 5392-40-5 | 7.8    | 7.8   |        |
| Di-(2-ethylhexyl) phthalate | 117-81-7 | 0.3  | 0.3   | 0.3    | 0.3    |
| α-Limonene | 5989-27-5 | 1.0    | 1.0   |        |
| Geranyl acetate | 105-87-3 | 6.0    | 4.0   |        |
| Indole-3-carbinol | 700-06-1 | 0.2    | 0.2   |        |
| Isoeugenol | 97-54-1 | 6.0    | 4.0   |        |
| Malonaldehyde | 24382-04-5 | 2.1    | 2.1   |        |
| Melamine | 108-78-1 | 2.7    | 2.7   |        |
| Pulegone | 89-62-7 | 1.0    | 1.0   |        |
| Red 2G | 2611-82-7 | 8.0    | 8.0   | 3.4    | 3.6    |
| α,β-Thujone | 76231-76-0 | 1.0    | 1.0   |        |
| trans-Cinnamaldehyde | 14371-10-9 | 1.0    | 1.0   | 1.0    | 1.0    |
| Urotropin | 100-97-0 | 1.0    | 1.0   |        |
| Zearalenone | 17924-92-4 | 1.8    | 2.0   |        |
| β-Mycene | 123-35-3 | 1.0    | 1.0   |        |

Number of compounds: 21

|       |       |       |       |
|-------|-------|-------|-------|
| 11    | 14    |       |       |

*At least one surrogate NOAEL was derived.
*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 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 and LOAELs from subchronic and chronic toxicity studies

At least two ratios were calculated for each compound and species: NOAEL<sub>sc</sub>/NOAEL<sub>c</sub> and LOAEL<sub>sc</sub>/LOAEL<sub>c</sub> based on the concentration of the test compound in feed, and/or NOAEL<sub>sc</sub>/NOAEL<sub>c</sub> and LOAEL<sub>sc</sub>/LOAEL<sub>c</sub> 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 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<sub>c</sub> 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 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   | 2.1   |       |       |
| 4-Methylimidazole         | 822-36-6| 2.0   | 2.0   |       |       |
| 5-Hydroxymethyl-2-furfural| 67-47-0 | 3.0<sup>a</sup> | 2.0   |       |       |
| Amarant                  | 915-67-3| 5.3<sup>a</sup> | 25.0  |       |       |
| Anthraquinone             | 84-65-1 | 4.0<sup>a</sup> | 4.0   |       |       |
| Azorubine                 | 3507-69-9| 4.7<sup>a</sup> | 2.9<sup>b</sup> |       |       |
| Butylhydroquinone         | 1948-33-0| 6.0<sup>a</sup> | 4.6<sup>b</sup> | 4.5<sup>b</sup> |       |
| Butylhydroxyanisole       | 25013-16-5| 0.9   | 1.1   |       |       |
| Citral                    | 5392-40-5| 0.7<sup>a</sup> | 1.0   |       |       |
| Curcumin                  | 458-37-7| 2.5   | 1.0   | 1.8<sup>b</sup> | 0.8<sup>b</sup> |
| Di-(2-ethylhexyl) phthalate| 117-81-7| 0.7<sup>a</sup> | 0.4   |       |       |
| β-Limonene                | 5889-27-5| 2.0   | 2.0   |       |       |
| Erythrosine               | 16423-68-0| 0.3   | 0.3   | 0.2   | 0.2   |
| Eugenol                   | 97-93-0 | 1.0   | 1.0   |       |       |
| Geranyl acetate           | 105-87-3| 6.0<sup>a</sup> | 4.0   |       |       |
| Indole-3-carbinole        | 700-06-1| 0.3<sup>a</sup> | 0.3   |       |       |
| Isoeugenol                | 97-94-1 | 1.0   | 1.0   |       |       |
| Malonaldehyde             | 24382-04-5| 3.6<sup>a</sup> | 2.5   |       |       |
| Melamine                  | 108-78-1| 0.3<sup>a</sup> | 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<sup>a</sup> | 1.0   |       |       |
| α,β-Thujone               | 76231-76-0| 3.0<sup>a</sup> | 2.0   |       |       |
| trans-Cinnamaldehyde      | 14371-10-9| 2.0   | 2.0   | 1.9   | 1.8   |
| Zearealenol               | 17924-92-4| 3.6<sup>a</sup> | 4.0   |       |       |
| β-Myrcene                 | 123-35-3| 1a<sup>a</sup> | 1a<sup>a</sup> | 1a<sup>a</sup> | 1a<sup>a</sup> |

Number of compounds 27 12 12 19 19

<sup>a</sup> At least one surrogate NOAEL was derived.
<sup>b</sup> These NOAELs and LOAELs were adjusted by the dose decrement factor of 1.6 (see Material and Methods section).

Fig. 1. Graphical illustration of subchronic to chronic NOAEL and LOAEL ratios in mice and rats (data from Tables 1 and 2).
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., 2015; 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 sub-chronic 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 (LD₅₀) 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 (LD₅₀ > 2000 mg/kg). Impact of chemical structure and chemical structure activity (chemicals grouped together): selected endpoints can influence the prediction of extrapolations across durations of exposure. | 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) | 25.6 (average ratio)                                                                                   | Pohl et al. (2010)         |           |
| ECHA/REACH, IUCLID database                                                    | Oral                                                                             | 1256                         |                     |                 | Acute (LD₅₀) 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., LD₅₀ above the classification threshold of 2000 mg/kg). | Gini 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 50% 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)  | (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                 |                 | 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)    |           |

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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.fraunhofer-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. 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. | Roberts et al. (2015) |
| Industrial chemicals | ECHA CHEM, eChemPortal | Oral, rats and mice | 305 | 28 d vs. 90 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. | 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) 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) |

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Table 4 (continued)

| Compound class           | Source of dataset                          | Application/Species | Number of compounds | Number of ratios | Outcome (as reported by the authors) | NOAEL/LOAEL ratio, GM (GSD) | Reference |
|--------------------------|--------------------------------------------|---------------------|---------------------|------------------|--------------------------------------|-----------------------------|-----------|
| Subacute vs. chronic     |                                            |                     |                     |                  |                                      |                             |           |
| Heterogenous             | RIVM’s Advisory Centre of Toxicology; IPCS, ATSDR |                     |                     |                  | 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                 |                  | 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 anahofer-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                 | 2.1                          | Zarn and O’Brien (2018) |

Subchronic vs. chronic

| Compound class           | Source of dataset                          | Application/Species | Number of compounds | Number of ratios | Outcome (as reported by the authors) | NOAEL/LOAEL ratio, GM (GSD) | Reference |
|--------------------------|--------------------------------------------|---------------------|---------------------|------------------|--------------------------------------|-----------------------------|-----------|
| Chemicals                | Industry data                              | Oral, various species | 33                  |                  | Subchronic vs chronic                | 2.2 (90th perc. 5.8)        | (Weil and McCallister, 1963) as interpreted by Kalberlah et al. (2002) |
| Heterogenous             | NTP studies                                | Oral (gavage), 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) |
| 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                 |                  | Subchronic vs chronic                | There was a significant correlation between the LOELs determined in subchronic studies | Spielmann and Gerbracht (2001) |

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Food and Chemical Toxicology 146 (2020) 111784

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 |
|------------------------------|--------------------------------------------------------|---------------------|---------------------|------------------|----------|--------------------------------------|-----------------------------|-----------|
| 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 (≥365d) | a) 2.25 (3.6) b) 2.28 (3.8) | Groeneveld 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.frGainhofer-repdose.de (Bitch 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 pharmaceuticals | ECHA, EFSA, WHO, IARC, ATSDR websites. Pharmaceuticals: Medicines Evaluation Board (MEB) of the Netherlands | 44 (34 chemicals, 10 pharmaceuticals) | NOAELs from the subchronic studies show a good correlation with the NOAELs and BMDs from the carcinogenicity studies. | Subchronic vs. chronic (carcinogenicity study) | Braakhuis et al. (2018) |
| Industrial chemicals, pesticides, pharmaceuticals | ECHA CHEM, TonRef, Hess DB, IMI eTOX, ELINCS, RepDose | a) Oral, rats and mice b) Inhalation, rats and mice | a) 462 b) 71 | 90 days (82–121 days) vs. ≥ 350 days | 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 (ESFA, 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.

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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 - Review & Editing. Dirk W. Lachenmeier: Validation, Writing - Review & Editing. Alexander T. Cartus: Validation, Writing - Review & Editing. Stephan H. Hüser: Investigation (data analysis and interpretation), Writing - Review & Editing. Matthias Baumb: 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.

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Appendix A. Supplementary data

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