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Derivation of the critical effect size/benchmark response for the dose-response analysis of the uptake of radioactive iodine in the human thyroid

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HIGHLIGHTS

- Intra-individual variation in the 24-h uptake of iodine by the thyroid is examined.
- For euthyroid adult subjects, a between-days difference of 20% is concluded.
- This inherent variation decreases the precision of relative RAIU data.
- A critical effect size (CES/BMR) of 20% is proposed for benchmark dose analysis.

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ABSTRACT

Potential adverse effects of chemical substances on thyroid function are usually examined by measuring serum levels of thyroid-related hormones. Instead, recent risk assessments for thyroid-active chemicals have focussed on iodine uptake inhibition, an upstream event that by itself is not necessarily adverse. Establishing the extent of uptake inhibition that can be considered de minimis, the chosen benchmark response (BMR), is therefore critical. The BMR values selected by two international advisory bodies were 5% and 50%, a difference that had correspondingly large impacts on the estimated risks and health-based guidance values that were established. Potential treatment-related inhibition of thyroidal iodine uptake is usually determined by comparing thyroidal uptake of radioactive iodine (RAIU) during treatment with a single pre-treatment RAIU value. In the present study it is demonstrated that the physiological intra-individual variation in iodine uptake is much larger than 5%. Consequently, in-treatment RAIU values, expressed as a percentage of the pre-treatment value, have an inherent variation, that needs to be considered when conducting dose-response analyses. Based on statistical and biological considerations, a BMR of 20% is proposed for benchmark dose analysis of human thyroidal iodine uptake data, to take the inherent variation in relative RAIU data into account. Implications for the tolerated daily intakes for perchlorate and chlorate, recently established by the European Food Safety Authority (EFSA), are discussed.

1. Introduction

Measurement of the uptake of radioactive iodine as a diagnostic tool for thyroid function testing (RAIU test) was introduced in the fifties of the previous century (Astwood and Stanley, 1947; Goodwin et al., 1951; Greer, 1951). As reliable methods for measuring thyroid hormones became available the diagnostic use of the RAIU test declined, but in recent years the test has been employed for examining the potential adverse effects of chemical substances on the human thyroid (Braverman et al., 2005, 2006; Greer et al., 2002; Hunault et al., 2007; Kunii et al., 2016; Lawrence et al., 2001, 2000). In the RAIU test, a quantity of radioactive iodide, usually sodium ^{131}I-, ^{132}I- or ^{123}I-iodide, is administered orally. At a specific time after administration, the fraction of the radioactivity absorbed by the thyroid is determined by measuring the locally

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emitted radiation by means of a probe held near the anterior neck (Becker et al., 1996).

The normal range of values for the 24-h iodine uptake in euthyroid subjects is usually defined as 10–30% but may be as large as 15–50% in certain parts of the world (Becker et al., 1996; Franklyn and Shephard, 2000). In the Greer et al. study, the range of 24-h RAIU values before treatment was 9.8–33.7% (Greer et al., 2002). The rather large normal range has implications for studies into the potential effects of a chemical substance on the thyroidal iodine uptake in humans, because it reduces the discriminating power of a dose-response study with a standard design (several treatment groups and a control group). That is, the relatively large inter-individual variability in iodine uptake makes it difficult to distinguish a change in RAIU due to exposure to a substance when comparing group average values of exposed and unexposed subjects.

To account for the impact of inter-individual variability in RAIU in the human population, several researchers have used study designs in which subjects served as their own controls and where the effect measure is the change in RAIU value during exposure to a substance relative to the subject's pre-study value (Greer et al., 2002; Lawrence et al., 2001; Lawrence et al., 2000). However, this procedure does not account for intra-individual variability and implicitly assumes that the subject's pre-treatment value is constant during the study, and ignores the normal day-to-day variation in thyroidal iodine uptake. This omission becomes critical if the calculated relative differences are used for deriving a tolerable daily intake level for a substance, employing benchmark dose (BMD) analysis.

BMD analysis is recommended by regulators and other scientists to assist the determination of, or to establish acceptable limits for, exposure for chemical substances (Crump, 1984; EFSA, 2009; U.S. EPA, 2012). A BMD analysis essentially involves fitting a dose-response curve to the observed study data (often obtained at higher doses and response levels than would be desired in the human population) and then using the mathematical function describing that curve to predict the dose or level of exposure that would be associated with a minimum level of a physiological response considered adverse. The minimum level of a physiologically adverse response (e.g., a level of iodine uptake inhibition, a change in body weight, a change in the serum concentration of a hormone) is termed the benchmark response (BMR). Proper selection of a BMR is critical to the BMD analysis; the BMR must be a meaningful change, both in terms of statistics (is the degree of change described by the BMR distinguishable from background?) and biology (is the degree of change meaningful in terms of being on the margin of something physiologically adverse?).

BMD analysis was conducted for assessing the health risk of exposure to perchlorate by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 2011 (FAO and WHO, 2011) and more recently by the Panel on Contaminants in the Food Chain (CONTAM) of the European Food Safety Authority (EFSA, 2014). Both expert groups established a tolerable daily intake (TDI) for perchlorate using data on thyroidal iodine uptake inhibition determined in the Greer et al. human volunteer study. JECFA selected a BMR of 50% for the inhibition of thyroidal iodine uptake, because this level of inhibition was not associated with changes in the levels of thyroid hormones or TSH following short-term and chronic exposure of healthy adults to perchlorate (Greer et al., 2002; Lamm et al., 1999; Lawrence et al., 2000; NAS, 2005). In contrast, the CONTAM panel posited that chronic adaptive changes in the thyroid to compensate for a sustained inhibition of the uptake of iodine could lead to thyroid disease, in particular in mildly to moderately iodine deficient people, even if thyroid function tests show no effect following short-term exposure to inhibitors. Based on this hypothesis, the EFSA panel selected 5% inhibition of the iodine uptake as the BMR in their BMD analysis of the Greer et al. data. The CONTAM panel also argued that 5% is the default BMR value applied for continuous data, although EFSA's guidance describes this default value in the context of animal studies, and indicates that health-endpoint-specific BMR values may be used based on statistical or toxicological considerations (EFSA, 2009). Largely because of this difference in BMR, the tolerable daily intakes established by JECFA and EFSA are different: 10 μg/kg bw/day versus 0.3 μg/kg bw/day, respectively.

Use of a default BMR value of 5% for change in RAIU does not appear consistent with the known variation in this parameter. Variation in RAIU measurement attributable to experimental errors is estimated to be ≤5% (Francois et al., 1958; Gomez Crespo and Vetter, 1966), but repeated measurements in euthyroid subjects (Francois et al., 1958; Hare and Haigh, 1955; Levy et al., 1959) revealed much larger differences between measurements in the same person. Francois et al. calculated a standard deviation of 15.3% for the relative differences of each of four 6-h RAIU measurements with the mean value of each subject (n = 17). The authors estimated that the physiological contribution to the observed intra-individual variation has a standard deviation of 14.4%, suggesting biological and/or environmental factors play a prominent role in the observed day-to-day variation of thyroidal iodine uptake. Irrespective of the cause of the variability, in order to decide what level of change is of toxicological relevance, the risk assessor needs to consider the normal variability for this endpoint in healthy individuals without known thyroid diseases. More specifically, when using the Greer et al. study for establishing a tolerable intake level for perchlorate, the inherent variation in the calculated IU inhibition due to the normal intra-individual inter-day variability in RAIU values in untreated subjects in the study population should be taken into account.

The present analysis was undertaken with the aim of establishing an appropriate BMR for the benchmark dose analysis of the inhibition of thyroidal iodine uptake in humans, in particular as determined in the Greer et al. study.

2. Materials and methods

Literature searches were conducted via MEDLINE (PubMed), and by searches on the Internet; the latest one in March 2016. Literature was selected primarily on the basis of the combinations of search terms 'thyroid', 'RAIU', 'radioactive', 'iodine/iodide' and 'variations' in the title or abstract. Publications cited in the selected articles were reviewed if considered appropriate. Only publications containing individual level RAIU data were included in the analysis. If data were only presented in graphical form, GetData Graph Digitizer (www.getdata-graph-digitizer.com) was used to retrieve data values. In the Greer et al. study, the 8-h and 24-h uptake of radioactive iodine was determined on several occasions. Several other studies were identified, in which 24-h radioactive iodine uptake was repeatedly measured in the same subjects, whereas no other repeated 8-h RAIU measurements were found in the literature. For this reason, we concentrated our analysis on available 24-h RAIU data.

As outlined in the introduction, in the Greer et al. study the effect of perchlorate on the thyroidal uptake of iodine was analysed by expressing RAIU values measured during treatment as a percentage of the pre-study RAIU value. To be consistent with this approach, we also analysed the untreated RAIU data obtained via the literature search using this approach, i.e., taking the first measurement collected as the baseline value:

$$\frac{|\text{first measure} - \text{second measure}|}{\text{first measure}} \times 100$$

We also conducted a sensitivity analysis comparing the absolute difference of the measurements to the second measure as well as the average of the two measurements. In cases where more than two measurements were collected, we calculated the maximum and minimum relative percent difference in RAIU values among the available measurements and then averaged the minimum and maximum results to produce one percent relative difference for each subject. Calculations were made in Microsoft Excel 2010.

Once an equivalent measure of variability in RAIU was obtained for each study, we pooled data from the various studies in order to calculate estimates of percentiles of RAIU variability as well as 95% confidence intervals (CIs) of estimates. We calculated estimates of the 1st, 5th, 10th, 25th, 50th, 75th, 90th, 95th, and 99th percentiles of the distribution of all measures of intra-individual RAIU variability and 95% confidence intervals for each estimate using the UNIVARIATE procedure in SAS version 9.3 (SAS Institute; Cary, North Carolina). This procedure calculates percentiles and CIs non-parametrically; that is, the method does not include an assumption of normality or any other distributional form. In addition, the procedure calculates coverage probabilities of each confidence interval. The nominal 95% CIs for estimated percentiles typically represent approximately 95% confidence that the true value falls within ("is covered by") the upper and lower bounds; however, 95% CIs for the percentiles near high and low extremes of a distribution may cover far less than 95% confidence on account of sparseness of observations in the tails.

3. Results

In addition to data from the study by Greer et al. (2002), three other studies were identified which provided individual level data on 24-h RAIU variability. Levy et al. (1959) studied the variation in iodine uptake on two subsequent days by taking 24-h RAIU measurements in 45 euthyroid subjects (Levy et al., 1959). The graphically presented data reveal a relative intra-individual difference in thyroidal iodine uptake of 0–83 percent on the second day. A second study (Hunault et al., 2007; Lambers et al., 2000) yielded data on a placebo control group of healthy adult volunteers (n = 10) in a 4-week toxicity study with nitrate; the 24-h RAIU was measured before and after the treatment period. The differences between the two measurements in the same volunteer range between 0 and 117% of the pre-study value. The third study identified was aimed at determining repeatability of 24-h RAIU measurements in 27 euthyroid subjects (Hare and Haigh, 1955). The authors reported the data of four measurements at weekly intervals in eight individuals, stating that these data are representative for the entire study group. The intra-individual differences between the 2nd, 3rd and 4th RAIU measurement and the 1st measurement range between 5 and 110%.

An untreated control group was not included in the Greer et al. study. However, the study authors concluded that the dose group average RAIU values determined two weeks after the end of treatment (P15) and the group average pre-treatment RAIU values (BL) were statistically indistinguishable, indicating complete recovery from the inhibiting effect of perchlorate. This is consistent with the very short biological half-life of perchlorate in humans, approximately 5 h (Tarone et al., 2010). For this reason, it is considered acceptable to compare the individual 24-h RAIU measurements taken in all 37 volunteers on those two days of the study. The absolute difference between the P15 and the BL value, calculated using the individual RAIU data from the Greer et al. study (Merrill, 2001), ranges between 0 and 72% of the BL value.

A considerable daily variation in thyroidal iodine uptake was also reported in a study in 17 healthy volunteers by Francois et al. (1958). These authors measured the 6-h RAIU on four days in the course of one week and found that the difference between the values measured on the 2nd, 3rd and 4th day and the RAIU value of the first day amounted 0–59%. Because this study involved shorter term measurements it was not used in our overall analysis.

We pooled the data from the four studies with 24-h RAIU data, resulting in a total sample size of 100 subjects (Fig. 1). The relative differences in RAIU ranged from 0 to 116.67% of the first RAIU measurement for each subject and had a mean and standard deviation of $18.58 \pm 19.58\%$. Estimates of percentiles and corresponding 95% CIs are displayed in Table 1. The nominal 95% CIs for the 1st and 99th percentiles have coverage probabilities far below 95% due to sparse data in the ends of the distribution. The precision of percentile estimates decreases as the percentile increases above the median. The 75th percentile of percent difference in RAIU is estimated to be 21.13% with fairly narrow confidence intervals (95% CI: 18.22, 39.50), while the 90th and 95th percentiles have much wider confidence intervals, indicating decreased certainty about the true value of these percentiles.

4. Discussion

Criteria have been proposed for assessing the reliability of an estimated benchmark dose (BMD) and its lower 95% confidence limit (BMDL) for risk assessment, and thus indirectly for the BMR chosen for the benchmark dose analysis. For instance, EFSA considers maximum BMD/BMDL and BMDU/BMDL ratios of 5 and 10, respectively, to be acceptable (EFSA, 2009; EFSA, 2015b). However, due to the fixation of the starting value (baseline value) at 100% in studies examining effects on thyroidal iodine uptake, the confidence interval of the BMD is necessarily smaller than for datasets from studies with an untreated control group. For this reason, the BMD/BMDL or BMDU/BMDL ratios are inadequate as reliability measures of the benchmark dose analysis of studies with this design. For a BMR to be an indication of adversity of a change in a physiological parameter, the distribution of the parameter values in the untreated control group have to be taken into consideration. The default BMR of 5% for continuous data in animal studies recommended by EFSA was among other things justified by referring to a re-analysis of a large number of studies in animals, which showed that the overall average $BMDL_{05}$ was close to the average of previously established NOAELs (EFSA, 2009). U.S. EPA

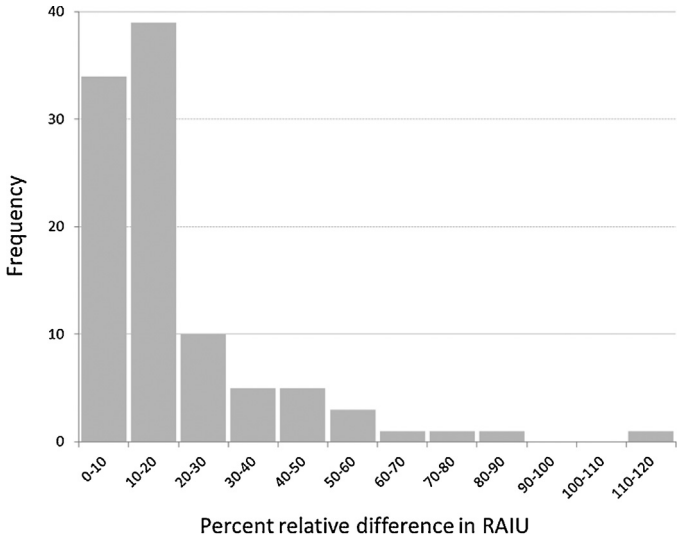

Fig. 1. Pooled results of four studies (Greer et al., 2002; Hare and Haigh, 1955; Lambers et al., 2000; Levy et al., 1959) in healthy euthyroid adults assessing intra-individual variability in iodine uptake (n = 100).

Table 1
Percentiles of relative differences in RAIU for pooled studies (n = 100 subjects).

Percentile	Estimate	95% CI	Coverage probability of 95% CIs
1	0	0, 0	55.46
5	0.61	0, 1.96	96.59
10	2.23	0.13, 3.61	95.23
25	4.82	3.71, 9.86	95.13
50	13.05	11.36, 16.80	95.4
75	21.13	18.22, 39.50	95.13
90	44.25	34.15, 69.30	95.23
95	57	45.00, 116.67	96.59
99	103.25	71.60, 116.67	55.46

prefers a biological basis for the BMR for continuous data, but has recommended using a change of one standard deviation from the concurrent control group mean value as the BMR as a default approach (U.S. EPA, 2012). These approaches for selecting a BMR have been proposed to account for the between-animal variation in untreated concurrent control groups. Distributions of the within-animal variation for biochemical and haematological control data from 36 studies were used by Dekkers et al. (2006) and Buist et al. (2009) for deriving BMR values. This approach was based on the assumption that temporal fluctuations in physiological parameters in healthy non-exposed animals are non-adverse. Nearly a quarter of the derived BMR-values were $\leq$5%, nearly a quarter were between 6 and 10%, a quarter was 15% or 20%, and nearly 30% of the BMR-values were $\geq$20% of the endpoint's control group mean value (Buist et al., 2009).

Our analysis of 24-h RAIU data in euthyroid subjects obtained from four studies (Greer et al., 2002; Hare and Haigh, 1955; Lambers et al., 2000; Levy et al., 1959) indicates that either the 75th percentile or the 90th percentile of the change in iodine uptake relative to the initial measurement would be the appropriate measure of the normal day-to-day change. The value at the 75th percentile is 21% (i.e., 21% change relative to baseline) while that at the 90th percentile is 44%. The 75th percentile value appears preferable to the 90th percentile because the 95% lower confidence interval on the former value (18%) is relatively close to the central tendency estimate (21%), suggesting the central tendency estimate is relatively stable. The confidence limits on the 90th percentile are substantially wider, suggesting less certainty in the central tendency estimate. It is also the case that a value associated with 75% of the normal range in biological response might be preferable from a precautionary perspective to a value associated with 90% (or even 95%) of the variable response. So as not to suggest too much precision in the estimates, the 75th percentile value could be rounded to a 20% change in iodine uptake relative to baseline as the normal day-to-day change in thyroidal iodine uptake. Consequently, a difference in RAIU, if measured in the same subject during exposure to an iodine uptake inhibitor, cannot be attributed to the inhibitor unless the difference with the pre-treatment value is more than 20%. To compensate for the inherent variability in the RAIU values during treatment, if expressed as a percentage of the pre-treatment value, it is proposed using this value as the BMR for benchmark dose analysis. Lower BMR values unavoidably result in biologically unreasonable benchmark dose levels.

In the scientific opinion on the risks to public health related to the presence of perchlorate in food, the EFSA CONTAM Panel selected a BMR of 5% to protect individuals in the population having a slight to moderate iodine deficiency (EFSA, 2014). The chronic iodine status in a population is inversely correlated with the avidity of the thyroid gland for iodine (Franklyn and Shephard, 2000; Keeling and Williams, 1972), but it is unlikely that the day-to-day variation in thyroidal iodine uptake is influenced by the long-term iodine status of a population. More likely, the observed large day-to-day variation in thyroidal iodine uptake is caused by daily variations in e.g. the diet, due to variable contents of iodine and (pre)goitrogenic substances (Grayson, 1960). Daily variability in iodine uptake in the mildly to moderately iodine-deficient population is therefore unlikely to be different from the similar variation we observed in four different studies (Greer et al., 2002; Hare and Haigh, 1955; Lambers et al., 2000; Levy et al., 1959). It may be that a prolonged marginal inhibition of thyroidal iodine uptake could potentially be adverse in the mildly to moderately iodine-deficient population as hypothesized by the EFSA CONTAM Panel (EFSA, 2014). In its opinion, the CONTAM Panel pointed out that other mechanisms than the hypothalamus-pituitary-thyroid feedback mechanism are available to the thyroid to compensate for a deficiency in iodine. The Panel argued that mild to moderate iodine deficiency can lead to the development of goitre and toxic multinodular goitre (TMNG). The underlying mechanism suggested by the CONTAM Panel is a combination of increased cellular proliferation (leading to thyroid growth) and an up-regulation of the production of intracellular hydrogen peroxide, bringing about an increased mutation rate, eventually leading to the autonomously functioning thyroid nodules that are characteristic for TMNG. The CONTAM Panel further postulated that exposure to substances interfering with the uptake of iodine at the sodium-iodine symporter (NIS) might aggravate the iodine status in the mildly to moderately iodine-deficient population leading to an increased prevalence of TMNG. Referring to JECFA's conclusion that prolonged 50% inhibition of thyroidal iodine uptake is without adverse effect, the EFSA CONTAM Panel suggested that this degree of inhibition may lead to goitre and TMNG, even if thyroid function is not changed during short-term exposure. We believe there are several weaknesses in the postulated mechanism. First, the hypothesis is based on epidemiological studies among smoking and non-smoking subjects in a mildly to moderately iodine-deficient population. In one of these studies (Knudsen et al., 2002), it was estimated that approximately 50% of goitre was attributable to smoking, but thiocyanate, the putative NIS inhibiting chemical originating from tobacco smoke, was not quantified. However, the CONTAM Panel assumed that smokers in this population had around 50% inhibition of thyroidal NIS, referring to another study (Laurberg et al., 2004), in which smoking was on average associated with a 50% decrease in the iodine content of breast milk. Thiocyanate was proposed by the authors as the cause of the lower breast milk iodine content, because blood levels of thiocyanate were higher in smokers than in non-smokers. However, an inverse correlation between perchlorate (a stronger inhibitor of iodine uptake than thiocyanate) and iodine in breast milk of lactating women was not found in four other studies (Dasgupta et al., 2008; Kirk et al., 2005; Leung et al., 2012; Pearce et al., 2007), which makes iodine uptake inhibition as the primary cause of the smoking-related increase in goitre less credible.

Secondly, the assumed absence of changes in short-term thyroid function tests is contradicted by the finding in recent large epidemiological studies that smoking is associated with a slight decrease in serum TSH and a slight increase in thyroid hormone levels, both in iodine-sufficient and iodine-deficient populations (Wiersinga, 2013). Which component of tobacco smoke is causing this small iodine-independent increase of thyroid hormone levels in smokers is not yet known, but it is unlikely thiocyanate, because an increase in thyroid hormone levels was observed after passive exposure to tobacco smoke for as brief as one hour (Metsios et al., 2007). This effect is opposite to what would be expected with competitive NIS inhibition and would also seem to be too short a window to deplete thyroidal iodine stores and trigger thyroid hormone changes. Tobacco smoke is a complex mixture, which complicates attributing physiological effects to a single component or to a single metabolite such as thiocyanate.

EFSA's hypothesis that goitrogenic substances such as thiocyanate and perchlorate may reduce the iodine content of the thyroid in the mildly to moderately deficient population even further is based on the positive association between mild iodine deficiency and the prevalence of multinodular goitre in Denmark. However, in this population, the iodine and selenium status are positively correlated and selenium deficiency has also been found to be associated with an increased prevalence of multinodular goitre (Rasmussen et al., 2011). In a population-based prospective cohort study in Germany, smoking was still positively associated with goitre in older subjects (≥60 years, with pre-existing goitre) after salt iodination, whereas in younger subjects (<40 years) an inverse association was found between smoking and the prevalence of goitre (Ittermann et al., 2008). This study shows that not only iodine deficiency but goitre is a pre-requisite for progression of the thyroid disease by smoking. These observations and studies render the suggested linear pathway from goitrogen-mediated iodine uptake inhibition to the aggravation of (toxic) multinodular goitre less probable. The observed association between smoking and (T)MNG in the mildly to moderately iodine-deficient population may be caused by components in tobacco smoke other than thiocyanate, and the underlying mechanism is probably more complex than merely inhibition of iodine uptake.

The cells of the thyroid also appear capable of managing short-term variations in iodine uptake and its metabolic consequences without adverse effects. In groups of mice, carrying a lacZ reporter plasmid, maintained on low iodine diets (intermediate and deficient) during one year, mild goitre developed after a few weeks, accompanied with decreased thyroid hormone but unchanged TSH. At 8 weeks and 3 months, antioxidant gene expression was increased, while the maximum expression of the TSH receptor gene and mRNA for NIS and thyroid peroxidase was measured at one year. The activation of antioxidant genes point at increased oxidative stress, probably caused by increased intracellular hydrogen peroxide. Increased thyroid DNA modifications were observed after 8 weeks (only examined in rats on an iodine-deficient diet). However, the mutation rate, which is much higher in thyroid cells than in other cell types, was unchanged, both in mice fed with the moderately iodine-deficient diet and in mice on the iodine-deficient diet. Apparently, the additional decrease in iodine intake was without any effect on the mutation rate (Maier et al., 2007). Human thyroid cells required much higher concentrations of hydrogen peroxide than T cells to induce detectable DNA damage, and cells survived radiation doses causing double-strand breaks (Versteyhe et al., 2013). These findings suggest that thyroid cells are particularly resilient against the effects of increased oxidative stress, such as brought about by a shortage of iodine, through up-regulation of anti-oxidant and glutathione peroxidase genes.

Summarising, the suggested role of thiocyanate in the association between increased prevalence of multinodular goitre and smoking in the mildly to moderately iodine-deficient population is unproven. The resilience of thyroid cells to oxidative stress and the stability of the mutation frequency under conditions of increasing iodine deficiency in the study by Maier et al. suggests that a slight inhibition of thyroidal iodine uptake in excess of the inhibition caused by mild to moderate iodine-deficiency is tolerated, providing a biological basis for a critical effect size larger than 5%.

However, our main argument against a BMR of 5% in benchmark dose analysis of relative RAIU data for human health risk assessment is of a statistical nature. Our analysis shows that the discriminative power of the study design does not allow a change smaller than 20% to be observed in the relative RAIU data. Thus, to take account of the inherent variation in secondary (relative) RAIU data, we propose selecting a BMR of 20% in the dose-response modelling of such data for deriving a tolerable daily intake.

It is reasonable to ask about the implications of slight goitrogen-associated changes in RAIU for a population of individuals even if these changes are within the range of temporal variation for the individual. We are familiar with such arguments being made in the past, notably with respect to lead and intelligence quotient (IQ). In the case of lead and IQ, it was argued that even non-clinically relevant changes in IQ at the individual level in response to lead exposure could be significant at the population level, particularly with respect to individuals in the 'tails' of the population response curve (Schmidt, 2013). Yet the situation with thyroid perturbation is fundamentally different. A decrease in IQ is an adverse outcome whereas a change in iodide uptake is not necessarily so. More importantly, IQ is understood to be an essentially fixed parameter and the only variation in measured IQ is due to limitations of the testing instrument. In contrast, as we have shown here, RAIU is highly variable over time and the normal temporal variation for a single individual accounts for a larger part of the measured value than the limits of the relevant test. Thus accounting for this intra-individual temporal variation in dose-response analysis is appropriate. Ideally that would be done by taking a large number of pre-exposure measurements in each subject to characterize that variability and testing the experimental results against that characterized baseline accordingly. However, the existing data were not obtained in this way and, in our analysis we attempt to correct for this deficiency.

5. Conclusions

We conclude that the inherent variability of the dataset should be taken into account when conducting benchmark dose analysis with secondary RAIU data from studies without a concurrent control group. In view of the normal intra-individual variability, a critical effect size (BMR) of 20% is considered most appropriate for benchmark dose analysis of these data.

In the case of perchlorate, using PROAST benchmark dose analysis software (see supplementary documentation), the resulting $BMDL_{20}$ is 16.6 μg/kg bw/day. Applying the assessment factor of 4, derived by the EFSA CONTAM panel to allow for interhuman differences in toxicokinetics (EFSA, 2014), leads to a TDI for perchlorate of 4 μg/kg bw/day. The TDI for chlorate, established by EFSA (EFSA, 2015a), is derived from the TDI for perchlorate and consequently may also need to be re-considered.

Conflict of interest

The authors declare that over the past five years they have conducted work for the fertilizer industry evaluating perchlorate's potential human health effects.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.toxlet.2016.06.004.

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