# Effects of cyanamide (CY) on the thyroid in relation to the proposed classification STOT RE1


## Summary

CY acts on the thyroid gland by inhibition of thyroid peroxidase (TPO) that may lead to a decrease of T3 and T4 in blood accompanied by an increase of TSH followed by stimulation of thyroid activity. Including TPO inhibition there are 5 different mechanisms of action (MOA) associated with the same sequence of effects on thyroid homeostasis. However, in all cases rats are by far more susceptible than humans and the sensitivity of rats and dogs is assumed to be comparable. The high sensitivity of rats and dogs in comparison to primates has specifically been demonstrated for sulfonamides, an important group of TPO inhibitors, and therefore the same species difference in sensitivity can also be inferred for CY. The high sensitivity of rats and dogs stems from their lack or limited amounts of the high affinity thyroxine binding globulin (TBG) the major transporter of T4 in primates acting as a “buffer” against fluctuations of thyroid hormones.

CY has been extensively used in the treatment of alcoholism and in more than 700.000 patients receiving average daily doses of 1 mg/kg bw/d over up to several years. No indications for clinically relevant disturbances of the thyroid became apparent. This NOAEL of 1 mg/kg bw/d in humans can be metabolically scaled to 4 mg/kg bw/d for rats, which is by a factor of 4 higher than the NOAEL obtained in the chronic study of the highly susceptible rat. This supports that the rat is not a suitable model to quantitatively predict thyroid effects in humans. On one hand for STOT classification reliable and relevant human data are preferable, on the other hand the trigger values for STOT 1 and 2 are referring to doses and effects noted in 90 day rat studies. Therefore this NOAEL, obtained by using a scaling factor of 4 between rats and humans, is used as more reliable starting point since rats and dogs are far more sensitive than humans. In addition if animal data are used for STOT classification they should show severe or significant toxic effects in the target organ in the dose ranges given and should be of relevance for humans. Although TPO inhibition is of relevance for humans, the dose levels causing effects in animal experiments are clearly misleading for an extrapolation to humans. From the experimental findings with CY, a scientifically justified, objective gradation of severity or significance of toxicity is very difficult and can be controversial. But the most severe consequence of chemicals affecting thyroid homeostasis, namely tumour induction, has not been observed in carcinogenicity studies with CY at the same dose levels as those used in 90 day and 2 year repeated dose studies.

In addition prolongation of exposure did not lead to a decrease of the NOAELs for rats and dogs.

For STOT classification, the broad database in humans and the high sensitivity of rats and dogs in comparison to humans should be taken into account. The epidemiological NOAEL in humans should be compared with the NOAEL in the chronic rat study using the metabolic scaling factor of 4 to extrapolate from humans to rats. Thereby it can be shown that humans are at least by a factor of 4 less sensitive than rats for effects on the thyroid gland. This difference in sensitivity must be taken into due account for extrapolating the experimental LOAELs to humans. It is shown that such extrapolated LOAELs are above the classification limit for STOT RE1 regardless of whether the effects are considered as “severe” or “significant”. Therefore STOT RE1 is not justified and STOT RE2 would be appropriate.

## Introduction

Davidson et al. (1979) have shown that CY is an inhibitor of thyroid peroxidase (TPO) activity similar to thioureylenes like thiourea, propylthiouracil or uracil. This was demonstrated in vitro with TPO preparations where CY inhibited the TPO catalysed peroxidation of guaiacol and iodide as well as the iodination of a tyrosine containing tripeptide. In vivo, doses of CY at 30, 50 and 100 mg/kg (once, ip) led to a massive dose related decrease of thyroidal protein iodination and a reduction of microsomal guaiacol oxidation (guaiacol oxidation is a standard assay for TPO activity).

The mode of action of TPO inhibitors is well established (McClain, 1995; Capen, 1997): iodination of the tyrosyl residues of thyroglobulin in the thyroid is inhibited as well as the coupling reaction of the iodotyrosines to finally form T3 and T4. This leads to a reduction of circulating T3 and T4 and by negative feedback on the pituitary to an increase of TSH. Thereby the thyroid is stimulated and reacts with increased weight and follicular cell hyperplasia that may eventually end up in thyroid tumours, as shown especially in the rat.

The relevance of effects observed after administration of CY to rats (and dogs) will be discussed under the following aspects:

1. Are the animal data described in the CLH report compatible with a TPO inhibition MOA as proposed by Davidson et al. (1979)?
2. Sensitivity of rats and dogs in comparison to humans
3. Sensitivity of rats and primates against sulfonamides
4. Observation in humans in relation to animal data
5. Consequences and proposal for STOT RE classification.

## 1. Are the animal data described in the CLH report compatible with a TPO inhibition MOA?

Under this aspect the findings in the repeated dose studies related to the thyroid are extracted from the CLH report with the NOAELs and the LOAELs.

***Osheroff (1988****), 28 d, rat, gavage (5, 10, 20, 40 mg/kg bw/d)*:

T3: at high dose decrease by ~28% (no statistics given) in females only

T4: at high dose decrease by ~28% in males and females (no statistics given)

TSH: at high dose increase in males and females by 100% (no statistics given)

Relative thyroid weight: significant increase at 40 mg/kg in males; numerical, non-significant increase in females.

Histopathology: at 10 mg/kg follicular cell hyperplasia with decreased colloid content and closely packed follicles in males; females less sensitive with effects starting at 20 mg/kg.

NOAEL: 5 mg/kg

Remark: sensitivity males > females for histopathological effects and relative thyroid weight. Histopathology more sensitive than thyroid weight.

***Til (1975)****, 90 d, rat, diet (0.5, 1.5, 4.5 mg/kg bw/d)*:

T3, T4, TSH: no data

No effects on thyroid weight

Histopathology: small follicular lumens without colloid with proliferating epithelial cells and interfollicular cells. Some indication for lower sensitivity of females.

NOAEL: 1.5 mg/kg bw/d based on histopathology

***Til (1982****), 90 d, dog, gavage (0.6, 2, 6 mg/kg bw/d)*:

T3: at the end of treatment indications (but no statistical significance) for reduced plasma T3 for both sexes by the lowest values found at 6 mg/kg bw/d

T4: at the end of treatment a dose dependent decrease in males (-38% at 2 mg/kg bw/d; -47% at 6 mg/kg bw/d)

TSH: no data

Thyroid weights: no effects

Histopathology: no data given

NOAEL: 0.6 mg/kg bw/d based on (numerically) decreased T3 and T4

Remark: plasma T3 relatively low at 2 and 6 mg/kg bw/d in both sexes; plasma T4 decreased in males at 2 and 4 mg/kg bw/d

***Osheroff (1989****), 1 y, dog, gavage (0.2, 1, 5 mg/kg bw/d):*

T3: assessed as being lower in the high dose group at week 52 without statistical significance, but this is only seen in females

T4: at week 52 significant decrease in males at high dose and numerical (but not significant) decrease in females. At week 13 numerical decreases without statistical significance.

TSH: no data given

Relative thyroid weight: significantly increased in females at high dose, no effect in males.

Histopathology: no effects

NOAEL (for thyroid): 1 mg/kg bw/d for females primarily based on relative thyroid weight and indications for decrease of plasma T3 and T4

***Osheroff (1991****), 2 y, rat, gavage (1, 2.5, 7.5 mg/kg bw/d)*:

T3: at week 92 statistically significant decrease at 2.5 and 7.5 mg/kg bw/d in males and in females at 7.5 mg/kg bw/d with a dose response relationship

T4: at week 92 statistically significant decrease in males at 7.5 mg/kg bw/d

TSH: no data

Thyroid weight: increase at 7.5 mg/kg bw/d in males (relative weights) and females (absolute weights)

Histopathology: reduced colloid with microfollicles in males at 2.5 and 7.5 mg/kg bw/d and females at 7.5 mg/kg bw/d

NOAEL: 1 mg/kg bw/d based (mainly) on histopathology

***Koeter (1986****), 2-generation study, rat, diet (20, 60, 180 ppm, corresponding to 0.81, 2.52, 7.55 mg/kg bw/d):*

T3: no data

T4: no data

TSH: no data

Relative thyroid weight: significantly increased in high dose F0 and F1 male and female parents; absolute weights slightly above controls

Histopathology: increased number of small follicles with cuboidal epithelium and reduction of colloid in high dose F0 and F1 male and female parents

NOAEL: ~2.5 mg/kg bw/d based on histopathology and organ weights

***Conclusion:*** These studies show consistently an increase of thyroid weights, indications of increased thyroid activity by histopathology, a decrease of serum T3 and T4 in rats and an increase in TSH. Similar effects are seen in dogs. Generally histopathology is most sensitive and T3 and T4 less, most probably due to relatively high inter-animal variations of the hormone measurements. Such effects are expected for substances affecting biosynthesis of thyroid hormones by inhibition of TPO. The NOAELs were about 1 mg/kg bw/d in the 1 year dog and the 2 year rat study with LOAELs of 5 and 2.5 mg/kg bw/d, respectively. For the 90 day rat study, the most critical one for differentiating between STOT RE1 and RE2, the NOAEL was 1.5 and the LOAEL 4.5 mg/kg bw/d, i.e. a difference of a factor of 3.

## 2. Sensitivity of rats and dogs in comparison to humans

Mechanisms influencing thyroid homeostasis can be divided into those affecting peripheral metabolism of thyroid hormones (inhibition of 5'-deiodinase, induction of liver phase II enzymes) and those with direct action on the thyroid ( blockage of iodine uptake, inhibition of hormone secretion, inhibition of TPO) (Capen, 1997). In spite of these primary differences they all lead finally to the same effects as the TPO inhibitors, i.e. decrease of T3 and T4 and increase of TSH. In all cases the sensitivity of rats is much higher than that of humans what resides in the same basic species differences known for many years (McClain, 1995, Capen, 1997):

- In humans and monkeys T4 binds to thyroxine binding globulin (TBG), pre-albumin and albumin with binding constants of $10^{-10}$, $10^{-7}$ and $10^{-5}$. Rodents lack TBG and binding occurs to pre-albumin and albumin with an affinity of 3-5 orders of magnitude lower. TBG acts as kind of a buffer for changes in circulating T4.

- Therefore the percentage of free T4 related to total T4 is less in humans than in rats.

- The rat without functional thyroid requires about 10 times more T4 (20 µg/kg) for full substitution than the adult human (2.2µg/kg) (Capen, 1997).
- Plasma half-life of T4 is considerably shorter in rats (12 h) than in humans (5-9 days).
- Serum TSH is about 25 times higher in rodents than in humans (McClain, 1995), and in male rats higher than in females (Capen, 1997).
- All these factors lead to a higher functional activity of the rat thyroid in comparison to humans (McClain, 1995), as supported by histology. The interior of the rat thyroid is comprised of small follicles with small amounts of colloid surrounded by a cuboidal follicular epithelium. Only in the periphery of the rodent gland large follicles with abundant colloid and flattened epithelial cells are found while in contrast such follicles uniformly comprise the normal human thyroid.
- Finally, this difference in functional activity is also reflected by the spontaneous incidence of thyroid tumours, being about 2% in male rats and on average 0.004% in humans.

Therefore the rat reacts with a much higher sensitivity to all goitrogenic stimuli than humans, regardless of the different mechanisms outlined above (Capen, 1997) and the associated excessive secretion of TSH will eventually lead to thyroid tumours. This has been described in detail e.g. for inducers of hepatic microsomal enzymes (Capen, 1997) as well as for sulphonamides (McClain, 1995; Capen, 1997), an important class of TPO inhibitors that are used as therapeutic agents in human and veterinary medicine.

Especially for TPO inhibitors (sulfonamides) Capen (1997) showed that humans and monkeys are resistant to their thyroidal action in comparison to the high sensitivity of rats and dogs. A limited literature search was carried out to substantiate that the regulation of thyroid hormones in dogs is comparable to that in rats. Capen (1997) semi-quantitatively indicated that binding to TBG is lower in dogs than in humans. More quantitative data show that dogs are clearly comparable to rats as the half-life of T4 is 12 h (Le Traon et al., 2008; van Dijl et al., 2014) and the substitution therapy with T4 for hypothyroidism is for dogs 20-40 µg/kg bw/d depending on the mode of application, the same as reported above for rats. Therefore dogs will react to TPO inhibitors like rats and are much more sensitive than humans.

Some further data substantiating the species differences in thyroid hormones are listed in table 1 all showing that humans markedly differ from rats and dogs.

Table 1: Comparison of thyroid hormones in rats, dogs and humans

| | Species (reference) | | |
|---|---|---|---|
| Parameter | Rats | Dogs | Humans |
| Serum T4 (µg/l) | 50 (Ca) | 14-22 (Pet, Ka)* | 43-111 (Wi)<br>46-120 (Pea)* |
| Free fraction of T4 (%) | | 0.102 (Fe) | 0.03 (Fe) |
| T4 total mean residence time (d) | | 0.54 (Fe) | 6.8 (Fe) |
| T4 clearance rate (l/kg/d) | | 0.24 (Fe) | 0.017 (Fe) |
| Serum T3 (µg/l) | 0.5 (Ca) | 0.43 (Fe)<br>0.78-0.98 (Ka, Pet)* | 0.5-2.0 (Wi) |
| Free fraction of T3 (%) | | 1.43 (Fe) | 0.3 (Fe) |
| T3 total mean residence time (d) | | 0.4 (Fe) | 1.5 (Fe) |
| T3 clearance rate (l/kg/d) | | 1.99 (Fe) | 0.32 (Fe) |
| TSH (mU/l) | 100 (Ca) | | 0.6-6 (Pea)<br>0.3-2.5 (Wi) |

*: for conversion of nmol/l the molecular weights of T4 (777) and T3 (651) were used

Ca: Capen (1997); Fe: Ferguson (2009); Ka: Kantrowitz et al. (2001); Pea: Peachey et al. (1989); Pet: Peterson et al. (1997); Wi: Wikipedia (2014)

**Sensitivity of rats and primates against sulfonamides**

While NOAELs and LOAELs have been established for the action of CY on the thyroid gland and the hormonal system in rats and dogs, no data are available for primates. But there is good reason for the assumption that primates are less sensitive to CY as deduced from the general mechanisms outlined above. Good evidence is especially obtained from data with sulfonamides that also act via TPO inhibition. According to Capen (1997) the goitrogenic effect of sulfonamides in the rat is known for over 50 years, and the dog is also a sensitive species in this respect. Examples showing the large quantitative difference by a factor 6-10 in the reaction of rats and primates against sulfonamides have been extracted from McClain (1995):

- Sulfamethoxazole: 50 mg/kg bw/d was goitrogenic in rats and produced thyroid neoplasia within 50 weeks. Treatment over 1 year at 300 mg/kg bw/d did not lead to effects on thyroid function or morphology in rhesus monkeys.

- Propylthiouracil and sulfamonomethoxine: Both substances given at 30 or 300 mg/kg bw/d led to decreased T3 and T4 concentrations and markedly elevated TSH accompanied by increased thyroid gland weight and follicular cell hyperplasia in rats. No effects on thyroid function or morphology were observed in monkeys at 300 mg/kg bw/d.

- Sulfamethazine: 600 ppm (approximately 30 mg/kg bw/d) was goitrogenic in rats and produced thyroid gland metaplasia. No effect on thyroid gland function was observed at 300 mg/kg bw/d over 13 weeks in cynomolgus monkeys.

Based on these findings it is reasonable to infer also for CY that humans are less sensitive than rats or dogs to effects on thyroid hormone homeostasis.

## 3. Observation in humans in relation to animal data

CY has been extensively used in treatment of alcoholism. The CLH report refers to more than 700,000 patients with a daily dose of about 1 mg/kg bw/d over approximately 100 days, but the duration of treatment may range from a few months to a few years, in some cases for more than 10 years. There are no indications for the induction of clinically overt hypothyroidism that should have become apparent in such patients under medical surveillance. Although subtle alterations of T3, T4 or TSH cannot be excluded, 1 mg/kg bw/d can be considered as a conservative NOAEL in humans for clinically relevant thyroid dysregulation.

These findings should be compared to the animal data. Without taking species differences in sensitivity to TPO inhibitors into consideration, metabolic scaling according to ECHA guidance R8 from humans to rats (factor 4) or dogs (factor 1.4) would be appropriate. Thus the NOAEL in humans would translate to a NOAEL for rats of 4 mg/kg bw/d and for dogs of 1.4 mg/kg bw/d. In both cases the experimental NOAELs in rats (e.g. by a factor of 4) and dogs are exceeded and the LOAELs are approached or also exceeded (for the 2 year rat study). The rat NOAEL as extrapolated from humans (4 mg/kg bw/d) still is below the classification limit for RE1 (10 mg/kg bw/d for 90 days), but it has to be considered that the "real" NOAEL for humans most probably is clearly higher. Apart from this, this comparison gives supporting evidence that humans are less sensitive than rats or dogs for the TPO inhibiting activity of CY.

## 4. Consequences and proposal for STOT RE classification

*General considerations*

For STOT RE classification the dose level leading to an adverse effect plays a dominant role in contrast to the pure hazard classifications, i.e. for carcinogenicity or mutagenicity. Therefore it has to be considered whether and to what extent the animal model may reflect the sensitivity of humans. If nothing is known in this respect, as in the majority of cases, the dose limits for RE1 and RE2 would apply. But for the thyroidal effects of CY there is strong evidence that humans are clearly less sensitive than the rat or the dog, although the extent is unknown.

For substances exerting their effect on thyroidal homeostasis by induction of liver microsomal enzymes the limited relevance of rat data for extrapolation to humans is well accepted. Thus the ECHA Guidance on the Application of CLP Criteria states (section 3.6.2.3.2, k, mode of action and its relevance to humans) that "certain thyroid tumours in rodents mediated by UDP glucuronyltransferase (UGT) induction" are caused by a mechanism "of tumour formation not relevant for humans". Reference is made to IARC (1999). While ECHA only refers specifically to the mechanism via induction of liver microsomal enzymes, IARC (1999) takes all mechanisms into consideration that act primarily via reduction of circulating thyroid hormones, including TPO inhibitors. Regarding species differences in thyroid carcinogenesis IARC (1999) finally concludes: "The weight of the evidence suggests that rodents are more sensitive than human subjects to thyroid tumour induction due to hormonal imbalances that cause elevated TSH levels". This assessment relating to tumour induction as the final consequence of hormonal imbalance by all mechanisms mentioned above is also valid for

the earliest precursors like increased weight of the thyroid gland or histopathological indications for its activation.

These assessments do not mean that humans may not respond in the same way as rodents at whatever high exposure level. Humans may principally react like the rat but at much higher exposures. This has been shown for the inducers of liver microsomal enzymes to which the ECHA guidance refers to. As an example, McClain (1995) mentioned anticonvulsant drugs which are enzyme inducing at therapeutic doses but only lead to mild changes in thyroid function of humans, i.e. moderately decreased T4 but normal T3 and TSH, changes that were not clinically significant. Similarly, rifampicin, a powerful enzyme inducer, only showed the same spectrum of a slight hormonal change in humans (Capen, 1997), i.e. no influence on T3 and TSH. This example for liver microsomal enzyme inducers shows that ECHA (in analogy to IARC) takes well into account quantitative differences in response between humans and rats, although qualitatively both react in the same way.

The same consideration would apply to CY. Its mode of action, i.e. TPO inhibition, is most probably also operative in humans. But due to the basic differences in rats, dogs and humans in hormonal regulation, humans are less sensitive than these experimental species. This leads to the basic question how this difference in sensitivity may translate into a differentiation between STOT RE1 and RE2.

*Comparison with the classification criteria*

For classification as STOT RE1 human data of “reliable and good quality evidence from human cases or epidemiological studies“ is required. More details are given in ECHA guidance R.7a stating that “reliable and relevant human data are preferable over animal data and can contribute to the overall Weight of Evidence“. And in addition: “negative human data cannot be used to override the positive findings in animals, unless it has been demonstrated that the mode of action of a certain toxic response observed in animals is not relevant for humans“.

Human data rely on about 700.000 patients under treatment for alcoholism. The strength of this data is the large population, the well-defined exposure and the long exposure duration. An important additional strength is the indication thereby obtained for the potential susceptibility of humans because the animal data do not allow an interspecies extrapolation. A weakness is the uncertainty about the intensity of the medical surveillance but symptoms for clinically relevant dysfunctions of the thyroid would certainly have been noted. Therefore it is proposed to take this data into account leading to an extrapolated NOAEL of 4 mg/kg bw/d

for rats thus indicating a difference in susceptibility of at least a factor of 4 between rats and humans based on the experimental NOAEL in rats of 1 mg/kg bw/day.

Animal data to be used should stem from “observations from appropriate studies in experimental animals in which **significant** and/or **severe** toxic effects, of relevance to human health, were produced at generally low exposure concentrations“. And category RE1 would be justified “when significant toxic effects observed in a 90-day repeated-dose study conducted in experimental animals are seen to occur at or below the guidance values”, i.e. ≤10 mg/kg bw/d. Besides the fact that the adverse effect (and not the NOAEL) should occur below the limit of 10 mg/kg bw/d (for a 90 day study) this definition comprises two important requirements for classification: the effects should be relevant to human health and should be significant or severe below the classification limit. This is also substantiated as it is stated in Annex I: 3.9.1.3 that “these adverse health effects include consistent and identifiable toxic effects … which have affected the function or morphology of a tissue/organ, or have produced **serious** changes to the biochemistry or haematology of the organism and these changes are relevant for human health“. Serious changes are further defined: “STOT-RE is assigned on the basis of findings of ‘**significant’** or ‘**severe’** toxicity. In this context ‘significant’ means changes which clearly indicate functional disturbance or morphological changes which are toxicologically relevant. ‘Severe’ effects are generally more profound or serious than ‘significant’ effects and are of a considerably adverse nature which significantly impact on health. Both factors have to be evaluated by weight of evidence and expert judgement”.

The relevance to human health has already been discussed above: A dysfunction of the thyroid certainly has a relevant and severe implication on human health and inhibition of TPO is a mechanism that may lead to such a dysfunction also in humans. But the total database shows that for the effects under consideration rats and dogs are poor models for any extrapolation always leading to clearly exaggerated risks for humans. Therefore the NOAEL of 4 mg/kg bw/d extrapolated from humans to rats should be the starting point. As the “true” extrapolated NOAEL would certainly be higher, the criterion “severity” or “significance” is not met by the epidemiological database.

As regards “severity” or “significance” of the effects, there are no objective criteria to decide whether or not the experimental findings are to be regarded as “severe” or “significant”. If an effect observed in a 90 day study would finally lead to tumour induction by prolongation of exposure, such an effect should be regarded as “severe” beyond any doubt. But tumour induction has not been observed in the carcinogenicity studies with CY: At exposures up to

7.5 mg/kg bw/d no thyroid tumours were found in the 2-year study of Osheroff (1991) with a group size of 20 animals/sex/dose. In the carcinogenicity study of Ulland et al. (1979) with 50 rats/sex/dose with technical calcium cyanamide (calcium cyanamide content 63%) in feed at dose levels of 100 and 200 ppm (males) and 100 and 400 ppm (females) no substance related increase of thyroid tumours was observed. According to the MAK value documentations (Vol. 24, 2007) these dose levels correspond to 5, 10 and 20 mg/kg bw/d of the test item or – taking into account the calcium cyanamide content of the test item and the difference in molecular weight - approx. 2, 3 and 6 mg/kg bw/d CY. Thus, the highest doses leading to thyroid related effects in a subchronic study (4.5 mg/kg bw/d) were not sufficient to result in a neoplastic response that would be the most relevant result for chronic administration of substances interacting with thyroid homeostasis in rats. Pre-stages leading to thyroid tumours after prolongation of exposure clearly would fulfil the criterion “severe”. But whether effects not associated with a neoplastic response in the highly sensitive rat should be regarded as “severe” or even “significant” cannot be decided on a scientific basis.

In the 90 day rat study (Til, 1975) the NOAEL was 1.5 mg/kg bw/d and the highest dose of 4.5 mg/kg bw/d led to histopathological alterations of the thyroid gland in 3/20 male and 2/20 female animals, while the thyroid weights were not affected. This effect cannot be regarded as “severe” taking into account the low incidence and that the thyroid weight was not affected. Of course it may be debated whether this finding is “significant”. Only at 7.5 mg/kg bw/d an increase of thyroid weight was observed in the 2 year (Osheroff, 1991) and 2-generation (Koeter, 1986) studies. This combination of effects may then be considered as “significant” or even “severe”.

But whatever effect in rats is taken to address the criterion “significant” or “severe”, the high sensitivity of this species in relation to humans has to be taken into account. By comparison of the experimental NOAEL in the rat (1 mg/kg bw/d in the 2 year study) with the evidence in humans it has been shown that rats are at least by a factor of 4 more sensitive than humans. Therefore it would be appropriate to apply this factor also to the effect levels in the rat studies. This would lead to an extrapolated LOAEL of 18 mg/kg bw/d for the 90 day rat study for an effect that may at most be regarded as “significant”. Similarly the effects at 7.5 mg/kg bw/d that might be regarded as “severe” would be extrapolated to 30 mg/kg bw/d. In both cases the classification limit of 10 mg/kg bw/d for STOT RE1 is exceeded and classification as STOT RE2 would be appropriate.

Further evidence for the notion that the effects observed in the 90 day studies are not to be regarded as “severe” can be derived from the comparison of the 90 day and 1 year studies in

rats and dogs: obviously the NOAELs do not decrease by prolongation of exposure nor were other potentially more severe effects noted at histopathology.

**Conclusion:** The broad database in humans and the high sensitivity of rats and dogs in comparison to humans should be taken into account. The epidemiological NOAEL in humans should be compared with the NOAEL in the chronic rat study using the metabolic scaling factor of 4 to extrapolate from humans to rats. Thereby it can be shown that humans are at least by a factor of 4 less sensitive than rats for effects on the thyroid gland. This difference in sensitivity must be taken into due account for extrapolating the experimental LOAELs to humans. It is shown that these extrapolated LOAELs are above the classification limit for STOT RE1 regardless of whether the effects are considered as “severe” or “significant”. Therefore STOT RE1 is not justified and STOT RE2 would be appropriate.

**References**

Capen CC (1997). Mechanistic data and risk assessment of selected toxic endpoints of the thyroid gland. Toxicol. Pathol. 25: 39-48

Davidson B, Soodak M, Strout HV, Neary JT, Nakamura C, Maloof F (1979). Thiourea and cyanamide as inhibitors of thyroid peroxidase: the role of iodide. Endocrinology 104: 919-924

Ferguson DC (2009). In Veterinary Pharmacologyand Therapeutics, 9th edition, Riviere, JE, Papich, MG Eds. ISBN: 978-0-8138-2061-3; Wiley-Blackwell. Section 8, 29. Thyroid Hormones and Antithyroid Drugs, p. 735 ff.

Greim, H (2007). The MAK-Collection for Occupational Health and safety, Part I. MAK value documentations, Vol. 24, Wiley-VCH: 41-49

IARC (1999). Scientific publication No. 147. Species differences in thyroid, kidney and urinary bladder carcinogenesis.

Kantrowitz LB, Peterson ME, Melian C, Nichols R (2001). Serum total thyroxine, total triiodothyronine, free thyroxine and thyrotropin concentrations in dogs with nonthyroidal disease. JAVMA 219: 765769

Le Traon G, Burgaud S, Horspool LJI (2008). Pharmacokinetics of total thyroxine in dogs after an administration of an oral solution of levothyroxine sodium. J. Vet. Pharmacol. Therap. doi: 10.1111/j.1365-2885.2007.00935.x.

McClain RM (1995). Mechanistic considerations for the relevance of animal data on thyroid neoplasia to human risk assessment. Mut. Res. 333: 131-142

Peachey JE, Annis HM, Bornstein ER, Maglana SM, Sykora K (1989). Calcium Carbimide in alcoholism treatment. Part 2: medical findings of a short-term, placebo-controlled, double-blind clinical trial. Brit. J. Addict. 84: 1359-1366

Peterson ME, Melian C, Nichols R (1997). Measurement of serum total thyroxine, triiodothyronine, free thyroxine, and thyrotropin concentrations for diagnosis of hypothyroidism in dogs. JAVMA 211: 1396-1402.

Van Dijl IC, Le Traon G, van de Meulengraaf BDAM, Burgaud S, Horspool LJI, Kooistra HS (2014). Pharmacokinetics of total thyroxine after repeated oral administration of levothyroxine solution and its clinical efficacy in hypothyroid dogs. J. Vet. Intern. Med. 28: 1229-1234

Wikipedia (2014). Schilddrüsenhormone; 08.11.2014