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SCIENTIFIC COMMITTEE ON PLANTS **SCP/PROPINEB/002-Final**
20 November 2001

OPINION OF THE SCIENTIFIC COMMITTEE ON PLANTS ON SPECIFIC QUESTIONS FROM THE COMMISSION REGARDING THE EVALUATION OF PROPINEB IN THE CONTEXT OF COUNCIL DIRECTIVE 91/414/EEC

(Opinion adopted by the Scientific Committee on Plants on 8 November 2001.)

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A. TITLE

OPINION OF THE SCIENTIFIC COMMITTEE ON PLANTS ON SPECIFIC QUESTIONS REGARDING THE EVALUATION OF PROPINEB IN THE CONTEXT OF COUNCIL DIRECTIVE 91/414/EEC

(Opinion adopted by the Scientific Committee on Plants on 8 November 2001)

B. TERMS OF REFERENCE

The Scientific Committee on Plants (SCP) is requested to respond to the following questions in the context of the Commission's work on the implementation of Council Directive 91/414/EEC concerning the placing of plant protection products on the market.

1. Can the Committee comment on the long-term exposure assessment for birds undertaken in the review?

2. Can the Committee comment on the appropriate animal model to be used for derivation of the Acceptable Daily Intake (ADI) and the Acceptable Operator Exposure Level (AOEL), considering the known hyper-sensitivity of rats to goitrogenic substances.

C. OPINION OF THE COMMITTEE

Question 1

Can the Committee comment on the long-term exposure assessment for birds undertaken in the review?

Opinion on question 1:

The Committee has identified a number of respects in which the risks from propineb to birds have not been adequately addressed. The Committee has also indicated a number of ways in which the risk assessment could be improved. The recommended approach is likely to produce lower TER's and might change the outcome of the risk assessment.

In addition the Committee is of the opinion that the long-term risk from propineb to wild mammals has not been adequately addressed. The long-term risk from metabolite PTU (propylenethiourea) for wild mammals should also be assessed.

The Committee recommends that attention be paid to the following points:
- Assessment of exposure for both birds and wild mammals should be based on daily dose rather than dietary concentration, allowance should be made for differences between dry and wet weight, and for differences in assimilation efficiency between different types of food;
- Assessment of whether effects on the thyroid should be considered a relevant end-point for wild mammals.

Finally, it is important to ensure that all the end-points, data, assumptions and rationale used for the risk assessment are clearly expressed and justified.

Question 2

Can the Committee comment on the appropriate animal model to be used for derivation of the Acceptable Daily Intake (ADI) and the Acceptable Operator Exposure Level (AOEL), considering the known hyper-sensitivity of rats to goitrogenic substances.

Opinion on question 2:

Propineb, probably through its metabolite propylenethiourea, has goitrogenic effects in rats, mice and dogs, rat being the more sensitive species because it is particularly vulnerable to disturbances in thyroid homeostasis. However, toxicity is expressed in rats also with effects on liver and kidneys that do not appear to be linked with thyroid effects. These liver and kidney effects in rats are seen at dose levels below those at which toxicity can be observed in either mice or dogs. Therefore, although the thyroid effects of propineb in rats are inappropriate for human risk assessment, the Committee considers that the rat remains an appropriate species for the derivation of ADI and AOEL.

A. TITLE

REPORT OF THE SCIENTIFIC COMMITTEE ON PLANTS ON SPECIFIC QUESTIONS FROM THE COMMISSION REGARDING THE EVALUATION OF PROPINEB IN THE CONTEXT OF COUNCIL DIRECTIVE 91/414/EEC

B. TABLE OF CONTENTS

C. BACKGROUND

Propineb is an existing active substance (a.s.) in the context of Council Directive 91/414/EEC[1], which is covered by the first stage of the work programme established by Commission Regulation (EEC) 3600/92[2].

A draft assessment report (monograph) has been prepared by the Rapporteur Member State (RMS, Italy) on the basis of a dossier presented by the notifier (Bayer AG). In order to prepare its opinion the Scientific Committee on Plants had access to this draft assessment report, the evaluation table and other documents as listed below.

Propineb is a fungicide of the dithiocarbamate family. It is used for the control of disease in a wide range of crops, including fruit crops and vegetables. It is not systemic. Its rate of use ranges from 1.05 to 7.0 kg a.s./ha /application. Taking account of the number of applications per season, up to 20 kg a.s./ha/ season can be applied.

[1] OJ N° L 230 of 19. 8.1991, p. 1.
[2] OJ N° L 366 of 15.12.1992, p. 10.

Source documents made available to the Committee:

1. Propineb: Terms of reference, submitted by DG Health and Consumer Protection, 2 May 2001 (SCP/PROPINEB/001).

2. Propineb: Evaluation table - 7575/VI/97 Rev. 11 (13/03/2001), submitted by DG Health and Consumer Protection, 2 May 2001 (SCP/PROPINEB/003).

3. Propineb: Annex to the Evaluation table – 25/01/2000, submitted by DG Health and Consumer Protection, 2 May 2001 (SCP/PROPINEB/004).

4. Propineb: List of end-points, submitted by DG Health and Consumer Protection, 2 May 2001 (SCP/PROPINEB/005).

5. Propineb: RMS comments on risk of propineb for predatory mites, submitted by DG Health and Consumer Protection, 2 May 2001 (SCP/PROPINEB/006).

6. Propineb: Comments from Austria, 20/12/1999 (Environmental fate & behaviour and Ecotox), submitted by DG Health and Consumer Protection, 2 May 2001 (SCP/PROPINEB/007).

7. Propineb: Comments from Belgium, 6/12/1999 (Mammalian toxicity), submitted by DG Health and Consumer Protection, 2 May 2001 (SCP/PROPINEB/008).

8. Propineb: Comments from Germany (operator exposure) submitted by DG Health and Consumer Protection, 2 May 2001 (SCP/PROPINEB/009).

9. Propineb: Comments from Sweden (long term risk for terrestrial vertebrates) submitted by DG Health and Consumer Protection, 2 May 2001 (SCP/PROPINEB/0010).

10. Propineb: Comments from Sweden (discussion on mammalian toxicity) submitted by DG Health and Consumer Protection, 2 May 2001 (SCP/PROPINEB/011).

11. Propineb: Comments from Sweden (further comments on long term risk for terrestrial vertebrates) submitted by DG Health and Consumer Protection, 2 May 2001 (SCP/PROPINEB/012).

12. Propineb: Comments from Sweden (further comments (2) on long term risk for terrestrial vertebrates) submitted by DG Health and Consumer Protection, 2 May 2001 (SCP/PROPINEB/013).

13. Propineb: Comments from Sweden (on long term risk for terrestrial vertebrates), submitted by DG Health and Consumer Protection, 2 May 2001 (SCP/PROPINEB/014).

14. Propineb: Comments from Denmark (metabolites and ground water contamination) submitted by DG Health and Consumer Protection, 2 May 2001 (SCP/PROPINEB/015).

15. Propineb: Comments from notifier (cover page) submitted by DG Health and Consumer Protection, 2 May 2001 (SCP/PROPINEB/016).

16. Propineb: Comments from notifier (list of end-points according to notifier) submitted by DG Health and Consumer Protection, 2 May 2001 (SCP/PROPINEB/017).

17. Propineb: Comments from notifier (Position paper for the classification of PU as a non relevant soil metabolite) submitted by DG Health and Consumer Protection, 2 May 2001 (SCP/PROPINEB/018).

18. Propineb: Draft review report, submitted by DG Health and Consumer Protection, 2 May 2001 (SCP/PROPINEB/019).

20. Propined: Draft assessment report prepared by Italy in the context of the inclusion of propined in Annex I to Council Directive 91/414/EEC (Volumes 1 to 3), June 1996.

21. Jones, R.D. (1999) Technical Grade Antracol: A Subchronic Toxicity Feeding Study in the Beagle Dog. Bayer Corporation Agriculture Division Report No. 108678, February 1999.

22. Brune, Deutsch-Wenszel (1980) 2 year mouse study on rats (chronic feeding study) – Study N° R 1792 (Property of Bayer AG).

23. Löser (1973) 2 year chronic toxicity in dogs (Bay 46131) – Study N° 4213 (Property of Bayer AG).

24. Löser (1974a) 2 year chronic toxicity study on rats (Bay 46131) – Study N° 4608 (Property of Bayer AG).

25. Löser (1974b) 2 year 2 year chronic toxicity study on rats (Bay 46131) – Study N° 4927 (Property of Bayer AG).

D. SCIENTIFIC BACKGROUND ON WHICH THE OPINION IS BASED

I. Question 1

Can the Committee comment on the long-term exposure assessment for birds undertaken in the review?

Opinion of the Committee:

The Committee has identified a number of respects in which the risks from propineb to birds have not been adequately addressed. The Committee has also indicated a number of ways in which the risk assessment could be improved. The recommended approach is likely to produce lower TER's and might change the outcome of the risk assessment.

In addition the Committee is of the opinion that the long-term risk from propineb to wild mammals has not been adequately addressed. The long-term risk from metabolite PTU (propylenethiourea) for wild mammals should also be assessed.

The Committee recommends that attention be paid to the following points:

– **Assessment of exposure for both birds and wild mammals should be based on daily dose rather than dietary concentration, allowance should be made for differences between**

dry and wet weight, and for differences in assimilation efficiency between different types of food;

- **Assessment of whether effects on the thyroid should be considered a relevant end-point for wild mammals.**

Finally, it is important to ensure that all the end-points, data, assumptions and rationale used for the risk assessment are clearly expressed and justified.

Scientific background on which the opinion is based:

I.1 Origin of question

The Committee looked for the origin of the question in the documentation provided and identified the following points of concern that were raised during the evaluation:

1. The confusing use of not comparable units (i.e. mg/kg food and mg/kg bw per day) in the tables presenting the toxicity exposure ratios.
2. Missing data for estimating the half-life of propineb in green mass. The RMS used a DT_{50}[3] of 43 hours for propineb in green mass but it was not clear how this was derived.
3. The question whether the risk of the metabolite PTU (propylenethiourea) should be addressed or not.

I.2 Hazard/risk assessment for exposure of birds and wild mammals

I.2.1 Confusing use of incomparable units

In some of the tables produced by the RMS, incomparable units (e.g. mg/kg food and mg/kg bw per day) have been used. However, the calculations of the TERs[4] are correct. The short term and the long-term risk assessments of the RMS are based on the unit mg/kg food. To avoid bias due to different food intake rates between laboratory and field and between small and large species the Committee recommends to base the risk assessment on daily doses. For estimating this daily dose it is recommended to correct for differences between dry weight and wet weight and for differences in assimilation efficiency for different food types.

I.2.2 Half-life of propineb in green mass

The RMS used a DT_{50} of 43 hours in green mass in the risk assessment. It is not clear how the RMS derived this value. In order to make the assessment more transparent it is advised to provide all essential data in the summaries that will enable other Member States or stakeholders to endorse the risk assessment carried out by the RMS. However, in view of the DT_{50}s for propineb in soil of less than 0.7 hours to 6 days (median 3 hours; 20°C, aerobic, n=6, according to SCP/PROPINEB/017) and all other possible routes of disappearance (e.g. hydrolysis, photolysis, volatilisation and dilution by growth) the order of magnitude seems to be reasonable.

I.2.3 Hazard/risk assessment of propineb and PTU

The RMS has made an assessment of the acute hazard/risk for wild mammals. In view of the relatively high TERs (>180) it was concluded that propineb represents a low acute risk to wild

[3] Period required for 50% dissipation.
[4] Toxicity Exposure Ratios.

mammals. In addition it was stated that in view of the relatively high rate of disappearance of propineb residues no significant long-term risk exists for wild mammals. However detailed justification for such a statement was not provided.

It is recommended that for propineb and its metabolite PTU an assessment be made for wild mammals of the possible effects on the thyroid and the results of these effects on the survival and reproduction rate of wild mammals, in relating the outcome in laboratory animals (in long-term laboratory studies) with wildlife exposure conditions.

Laboratory and field evidence suggests that birds, unlike mammals, are not susceptible to xenobiotic-induced changes to thyroid function (Dawson, 2001).

I.2.4 Wider issues affecting the assessment

The procedure in the EU for estimating the potential risk for long-term exposure of birds is normally based on a comparison of the contaminated food source (expressed in mg/kg food) and the no observed effect concentration which is also expressed in mg/kg food. However, more scientifically justifiable toxicity exposure ratios would be based on daily dose in order to avoid bias due to different food intake rates between laboratory and field and between small and large species.

Very often in risk assessment a simple approach is followed: small birds and wild mammals (less than 100 grams) will consume a dry weight of food equivalent to 30% of their (wet) body weight, and larger birds and wild mammals will consume 10% of their body weight. The resulting consumption estimates should be converted to wet weights before multiplying by pesticide concentration to calculate the dose. Frequently, the correction to wet weight is omitted, which causes under-estimation of the dose.

A more accurate approach was described by EPPO (1994, p. 54). This approach is based on the allometric relationships between the daily food intake (grams/day) and the body weight of birds and wild mammals published by Nagy in 1987. Also in this case a correction has to be made for the differences between dry weight and wet weight. In addition it is sometimes necessary to correct for the assimilation efficiency, especially when fruit and leaves are the appropriate food source to consider. Guidance can be found in Traas *et al.* (1996) and Jongbloed *et al.* (1996).

The normal procedure for estimating the initial residue levels on food ingested by birds and wild mammals in the EU is based on Hoerger and Kenaga (1972). In most cases the so-called "typical" values are used (the typical values are the mean values of the maxima for each crop/pesticide combination). Recently several studies have been carried out to check whether the results of the research of 1972 are still valid (different substances, low volumes, etc.) and to provide better data for small and large insects. These studies have been summarised by Luttik (2001) and new residue values have been calculated for different categories of food for birds and wild mammals.

I.3 General advice to increase the transparency of hazard/risk assessment.

The Committee has the following general advice to increase the transparency of the hazard/risk assessment:

- To produce a list of end-points and explain why each end-point is considered to be relevant or not relevant for the environmental hazard/risk assessment,
- To carry out a tiered approach and to provide all calculated TERs, even when it is evident for the RMS that the risk will be low,
- To underpin, in case refinement of the earlier tiers is appropriate, the assumptions that have been made (e.g. interception, time spent in the area, fraction of food type in diet, measured residue values instead of standard residue values, actual DT_{50}s instead of default DT_{50}, avoidance, etc.),
- In order to make the whole assessment more transparent it is also advised to provide all essential data in the summaries that will enable other Member States or stakeholders to endorse the risk assessment carried out by the RMS.

I.4 Conclusion

The Committee has identified a number of respects in which the risks from propineb to birds have not been adequately addressed. The Committee has also indicated a number of ways in which the risk assessment could be improved. The recommended approach is likely to produce lower TERs and might change the outcome of the risk assessment.

In addition the Committee is of the opinion that the long term risk to wild mammals has not been adequately addressed.

II. Question 2

Can the Committee comment on the appropriate animal model to be used for derivation of the Acceptable Daily Intake (ADI) and the Acceptable Operator Exposure Level (AOEL), considering the known hyper-sensitivity of rats to goitrogenic substances?

Opinion of the Committee:

Propineb, probably through its metabolite propylenethiourea, has goitrogenic effects in rats, mice and dogs, rat being the more sensitive species because it is particularly vulnerable to disturbances in thyroid homeostasis. However, toxicity is expressed in rats also with effects on liver and kidneys that do not appear to be linked with thyroid effects. These liver and kidney effects in rats are seen at dose levels below those at which toxicity can be observed in either mice or dogs. Therefore, although the thyroid effects of propineb in rats are inappropriate for human risk assessment, the Committee considers that the rat remains an appropriate species for the derivation of ADI and AOEL.

Scientific background of which the opinion is based:

II.1 Toxicology of propineb

Orally administered propineb in rats is rapidly absorbed and excreted, largely via urine and faeces. A proportion of the administered dose accumulates temporarily in the thyroid in this

species. Although most elimination occurred within 4 days of dosing, the half-life of elimination for the proportion remaining was relatively long.

Propineb has a goitrogenic effect in rats, mice and dogs, but no similar effect was noted in rabbits (albeit in a three-week study**,** Milhail & Kaliner, 1979, Bayer report 8322). A basis for evaluating thyroid effects in rats is in an annex to this opinion.

In a 62-day study of thyroid function in male rats, the NOAEL[5] was 10 ppm, equal to 0.74 mg/kg bw/day, based on changes in thyroxin concentration and increased thyroid weight at 50 ppm. Comparison with the effects of propylenethiourea, a primary metabolite in mammalian tissues (the other primary metabolite being propylenediamine) suggests that the goitrogenic effects of propineb are due to this metabolite.

Propineb has been tested for genotoxic activity in a suitable range of assays that have produced mainly negative results. A positive result was obtained in a mitotic gene conversion assay and "inconclusive" results were obtained in a bacterial DNA damage assay and a rat bone marrow chromosomal aberrations assay. Propylenethiourea also produced mainly negative results, but an "inconclusive" result was obtained in a bacterial DNA repair assay.

II.1.1 Sub-chronic toxicity studies

The sub-chronic, oral toxicity of propineb has been studied in 90-day experiments in rats (1 test) and dogs (3 tests).

Groups of Wistar rats (15/sex/group) were given diets containing propineb (purity not stated) at concentrations of 0, 5, 10, 25, 50 or 100 ppm for 90 days. This study was not considered acceptable because of important omissions in haematology and blood chemistry and the lack of ophthalmology and histopathology. There was no treatment-related mortality or clinical signs, but there were increased activities of lactate dehydrogenase at 100 ppm and sorbitol dehydrogenase in male rats at 50 ppm. Based on this latter measurement, the NOEL[6] was 25 ppm, equivalent to 1.25 mg/kg bw (Löser, 1969).

Of the three available 90-day oral studies in dogs, only the most recent study will be considered (Jones, 1999); it also provides the lowest NOEL. Beagle dogs (4/sex/group) were given diets containing propineb (82.1 – 82.9% purity) at concentrations of 0, 150, 1500 or 5000 ppm for 90 days. Additional groups of 3 males/dose group were used to study recovery during at least 4 weeks subsequent to treatment. Decreases in body weight occurred in the 5000 ppm group, but there was a strong recovery when treatment ended. No clinical signs of toxicity, ophthalmological changes, alterations in ECG or blood pressure were observed. Neurological changes were observed at 1500 and 5000 ppm, but the recovery group dogs returned to normality by the end of the study period. These findings (propreoceptive deficit and hind-limb wheel-barrowing) were consistent with canine hypothyroidism. Changes found in the 1500 and 5000 ppm groups included increases in liver and thyroid weights, increased T_3, TSH, cholesterol and globulin, decreased RBCs[7], haemoglobin, haematocrit and MCHC and increased MCV, MCH and percentage of reticulocytes. The NOEL was 150 ppm, equal to 4.3 mg/kg bw, based on thyroid-related toxicity at 1500 ppm (Jones, 1999).

[5] No Observed Adverse Effect Level.
[6] No Observed Effect Level.
[7] Red Blood Cells.

II.1.2 Chronic toxicity studies

The chronic toxicity of propineb has been studied in two-year dietary experiments in mice (1 test), rats (2 tests) and dogs (1 test).

In one of the rat studies, groups of Wistar rats (40/sex/group) were given diets containing propineb (86.2 – 86.7% purity) at concentrations of 5, 10, 25, 50 and 100 ppm for 2 years. The control group consisted of 95 rats/sex. There were no adverse effects of treatment on survival, body weight gain, clinical signs, haematology or blood chemistry except for, at 50 and 100 ppm, slight increases in GOT, particularly after one year, which nevertheless remained within the physiological ranges after two years. Protein-bound iodine was significantly reduced at 100 ppm after two years in both male and female rats. No treatment related effects on pathology or organ weights were observed (Löser, 1974a).

In the second rat study, groups of Wistar rats (25/sex/group) were given diets containing propineb (93.5% purity) at concentrations of 1, 10, 100, 1000, 2000 and 8000 ppm for 2 years. The control group consisted of 50 rats/sex. Mortality was increased in the 1000 ppm group from (males) 30% to 56% and (females) 26% to 76% and body weights were significantly decreased in this group from 398 g to 346 g in males and from 287 g to 149 g in females. Significant increases were found in the weights of liver and kidneys in males fed 100 ppm and there was a slight increase in blood GPT activity in females fed 100 ppm (GOT not measured in this study). Thyroid weights were increased in males fed 100 ppm and females fed 1000 ppm and there was an increase in thyroid tumour incidence amongst males from 3/34 (9%) in the controls to 6/15 (40%) in the 1000 ppm group. The NOEL was 10 ppm, equivalent to 0.5 mg/kg bw/day, based on increased weights of liver, kidney and thyroid of males at 100 ppm (Lőser, 1974b).

NMRI mice (50/sex/group) were given diets containing propineb (82.9% purity) concentrations of 0, 50, 200 and 800 ppm for 2 years. There were no adverse effects of treatment on survival, body weight gain, clinical signs, haematology or blood and liver chemistry. At autopsy, no treatment related abnormalities were found, but there were increased thyroid weights in females and an increased incidence of hepatocellular adenomas in males: controls: 3, 800 ppm: 9. The NOEL for this study was 200 ppm, equal to 26 mg/kg bw/day based on hepatocellular adenomas in male mice (Brune, Deutsch-Wenzel, 1980).

Beagle dogs (4/sex/group) were given diets containing propineb (86.2 – 86.7% purity) at concentrations of 0, 100, 300, 1000 or 3000 ppm for two years. There were no adverse effects of treatment on survival, body weight gain, clinical signs, haematology or ophthalmology. There was a decrease in blood alkaline phosphatase in females, but not in males, at 3000 ppm. The NOEL for this study was 1000 ppm, equivalent to 25 mg/kg bw/day based on decreased food intake by females at 3000 ppm (Löser, 1973).

II.2 Conclusions

Toxicity of propineb is expressed in rats also with effects on liver and kidneys that do not appear to be linked with thyroid effects. These liver and kidney effects resulted in NOAEL values in both subchronic and chronic experiments lower than those indicated by the experiment with mouse and dog. It should be noted that the NOAEL difference between rat and dog in the subchronic testing is rather small (a factor of 3), while in the chronic studies the difference is of a factor of at least 10. Therefore, although the thyroid effects of propineb in rats are

inappropriate for human risk assessment, the Committee considers that the rat remains an appropriate species for the derivation of ADI and AOEL.

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F. ANNEX: BASIS FOR EVALUATING THYROIDS EFFECTS IN RATS

1. The Thyroid – General.

The thyroid is unusual in two ways: it is functionally dependent on the dietary intake of a single element, iodine, and it is the main epithelial component embryologically derived from endoderm. Thyroid hormones regulate the metabolism of virtually all body organs and they are essential for growth and development. Hence, disruption of the function of this endocrine organ can have wide-ranging consequences. Hypersecretion leads to thyrotoxicosis and under-

secretion leads to myxoedema. Congenital hypothyroidism results in cretinism. Thyroid hyperplasia, also known as goitre, may be caused by autoimmunity or a dietary deficiency in iodine, while thyroid neoplasia develops predictably in rats particularly, but also in mice as a result of exposure to any procedure that induces prolonged and excessive secretion of thyroid stimulating hormone (TSH) (Atterwill & Cockburn, 1997).

1.1 *Human thyroid neoplasia*.

Benign thyroid tumours are relatively common in human, while carcinomas are uncommon. The great majority of the cancers are of follicular cell origin and give rise to differentiated (about 90%) and undifferentiated (anaplastic) carcinomas, the incidence rates being higher in women than in men. The differentiated carcinomas are either papillary or follicular, a distinction based on cytology, encapsulation, distribution of metastases and molecular biology. Small papillary carcinomas (formally known as occult tumours), or microcarcinomas, are quite frequent, but are of little clinical significance. Medullary carcinomas are derived from parafollicular, calcitonin-producing cells and form 5 – 15% of most series of thyroid carcinomas (Franceschi *et al.*, 1993).

Ionising radiation is the only clearly defined risk factor for thyroid carcinoma. No non-radioactive chemical exposure has been shown to result in development of thyroid carcinoma in humans. There is, furthermore, no convincing evidence to link thyroid cancer with areas of iodine deficiency (Hard, 1998). A programme of supplementation of food items with iodine in Sweden has not affected thyroid cancer trends in iodine-rich and iodine-deficient areas (Pettersson *et al.*, 1996). It is likely that the goiterogenic effects of chemicals involve mechanisms that are qualitatively common to all mammalian species, including humans. The sulfonamides (which include the thiourea metabolites of several pesticides) might appear to provide exceptions, since rats are sensitive to their goiterogenic effects, while no such effects are observed at high doses in monkeys or at therapeutic doses in humans. This separation of rats from primates is, however, most probably quantitative and due to marked species differences, in this case, in the inhibition of thyroid gland peroxidase.

1.2 *Rodent thyroid neoplasia.*

In untreated rats and mice aged two years or more, the incidence of thyroid follicular cell adenomas and carcinomas combined is about 1 – 3%, the incidence in male rats and male and female mice being higher than in female rats. Unlike the human neoplasms, no pathological and molecular biological division has been made into papillary and follicular types. In contrast to the general lack of evidence for a role of chemicals in human thyroid carcinogenesis, a large number of chemicals, including genotoxic substances such as *N*-nitrosoalkylureas and *N*-nitrosamines, have produced thyroid tumours in rodents (Dybing & Sanner, 1999; Wilbourn *et al*., 1999).

In a review of the 836 chemicals, mixtures, biological and physical agents, life-style factors and exposure circumstances evaluated within the IARC Monographs Programme, Vols. 1 – 69 (Wilbourn *et al*., 1999), 20 compounds induced thyroid follicular cell tumours in rats and/or mice. None of these is unequivocally associated with human thyroid cancer. Seven of the chemicals produced increases in thyroid tumours only in rats, two only in mice.

Amongst 240 pesticides evaluated by the US Environmental Protection Agency, Office of Pesticides Program Cancer Peer Review Committee, 37 were judged to induce effects on thyroid follicular cells amongst which 27 were judged to produce thyroid follicular cell tumours in

rodents (Hurley *et al.*, 1998). Three of these 27 pesticides induced these thyroid effects only at excessively high doses. Of the residual 24 pesticides, 22 induced thyroid tumours only in rats; none induced tumours exclusively in mice. The two substances producing effects in both rodent species were amitrole and ethylenethiourea. Neither propineb nor its presumed toxicologically active metabolite propylenethiourea, were in the list of pesticides evaluated. The Scientific Committee on Plants has responded to questions concerning two previously evaluated pesticides that had an action on the thyroid. These are amitrole (SCP/AMITR/002[8]) and isoxaflutole (SCP/ISOXA/012[9]). The latter increased the incidence of thyroid follicular cell tumours in rats and liver tumours in rats and mice.

2. Thyroid biochemistry

The function of the thyroid gland is regulated via a negative feedback process in which thyrotropin-releasing hormone (TRH) from the hypothalamus and thyroid-stimulating hormone (TSH) from the pituitary gland are released in response to decreased circulating levels of iodothyronines. This system of interacting hormones constitutes the *hypothalamic-pituitary-thyroid axis*.

Thyroid cell growth and function are regulated by a complex interaction of endocrine, paracrine and autocrine factors, the effects of which are mediated by a number of second messenger systems. Thyroid stimulating hormone (TSH) is the main growth factor for thyroid cells, maintaining the differentiated state of the thyroid and controlling thyroid hormone secretion. Other growth regulators involved in the system include insulin-like growth factor-I, epidermal growth factor, basic fibroblast growth factor, transforming growth factor β and an endogenous iodide-dependent mechanism (reviewed by Hard, 1998).

2.1 *Biosynthesis, transport and biologic action of iodothyronines*

The thyroid gland produces various iodine-containing compounds, iodothyronines, of which only four are biological active: thyroxine (T_4), triiodothyronine (T_3), tetraiodothyroacetic acid and triiodothyroacetic acid. The latter two are produced in such small amounts that they contribute little, if at all, to thyroid hormone action.

The synthesis and secretion of iodothyronines takes place in three main steps:

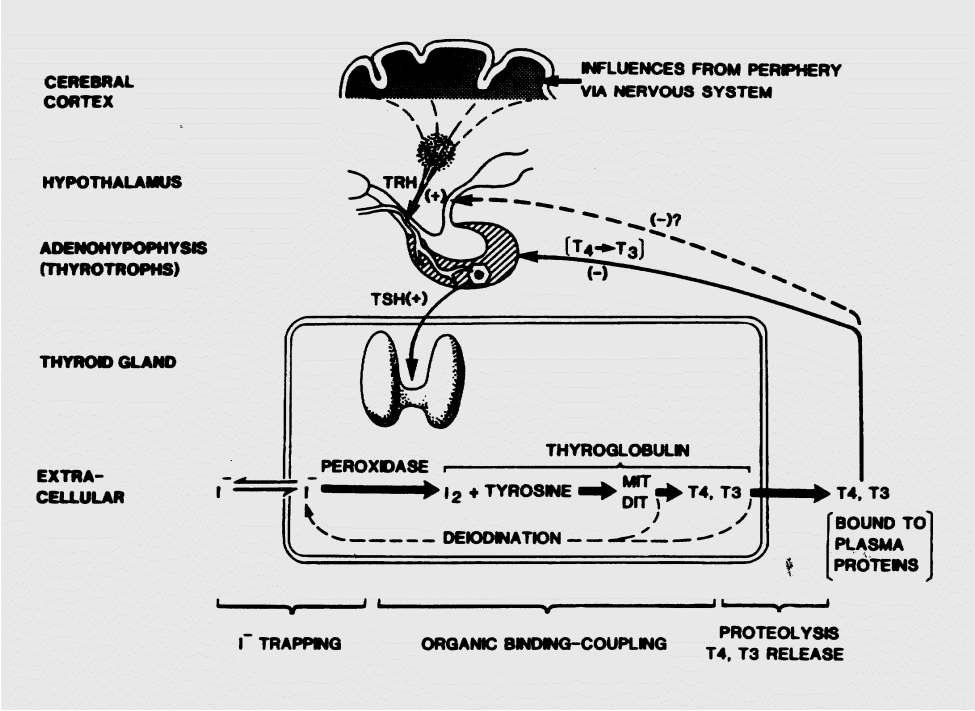
1. *Thyroid iodide uptake.* The first step in the synthesis of iodothyronines is uptake of iodide into the thyroid follicular cells. This is an energy-dependent process that, under normal conditions, concentrates iodide by up to about 30-50-fold its concentration in blood.

2. *Iodothyronine biosynthesis.* In the second step, iodide is oxidised by thyroid peroxidase (TPO) to reactive iodine that binds to tyrosyl residues in the thyroglobulin, resulting in the formation of mono- and di-iodotyrosines. The iodothyronines thyroxine (T_4) and triiodothyronine (T_3) are formed by coupling of these iodotyrosines.

3. *Iodothyronine release*. In the third step, the active hormones are enzymatically cleaved from the thyroglobulin and free T_4 and T_3 are released from the colloid into the blood circulation.

[8] http://europa.eu.int/comm/food/fs/sc/scp/out70_en.html
[9] http://europa.eu.int/comm/food/fs/sc/scp/out40_en.pdf

These steps and the hormonal interactions are shown diagrammatically below (after Capen & Martin, 1989).



T_4 is produced only by the thyroid gland, whereas the T_3 is produced primarily by extra-thyroidal mono-deiodination of T_4; the remainder comes from the thyroid gland. In human, about 80% of the circulating T_3 is derived by deiodination of T_4, while the thyroid secretes the remaining 20 %. In rats, the origin of T_3 is less well established, but it has been suggested that intrathyreoidal conversion of T_4 to T_3 provides the major source of T_3 in this species (Hard, 1998). The extra-thyroidal conversion of T_4 to T_3 mainly takes part in the liver and kidney, but also in brain, pituitary and brown fat. The thyroid gland contains large amounts of iodothyronines incorporated in thyroglobulin, the protein within which the two hormones are synthesised and stored as colloidal material in the follicular lumina. Because of these stores, T_4 and T_3 can be secreted rapidly when stimulated to do so without the need for *de novo* hormone synthesis.

2.2 *Species differences in goiterogenic effects, thyroid physiology and histology.*

There are several species differences in thyroid physiology. Thyroxin-binding globulin (TBG) is the predominant plasma protein in human and non-human primates that binds and transports thyroid hormone, T_4, in blood. This protein has binding affinities 3 and 5 orders of magnitude greater than the other two thyroxin-binding proteins, albumin and transthyretin (formally known as pre-albumin), respectively. In human, TBG is the major carrier protein for T_4 and T_3; transthyretin accounts for 20%, while albumin, through non-specific binding, carries only 10% of the circulating hormone. Collectively, these three proteins transport more than 95% of T_3 and T_4 in human (Bartalena & Robbins, 1992; Barlow, 1997). Free and bound T_3 and T_4 are in dynamic equilibrium in blood, there being approximately 0.4% of total T_3 and 0.04% of total T_4 that is unbound in human (Atterwill & Aylord, 1995). In rats, TBG occurs only as a postnatal surge, declining to nondetectable levels by early maturity at 8 weeks of age, but reappearing in senescence. The lack of TBG in rats throughout most of their lives is an important difference from the primates. In rats, transthyretin is the major carrier protein for T_4, but T_3 is carried only by albumin. An important function of the carrier proteins is believed to be extrathyroidal storage

of thyroid hormones as a mechanism to control release, thereby protecting target tissues from excessive fluctuations in exposure to the hormones. Probably as a result of the absence of a major high-affinity binding protein in rats, targets such as the thyrotrophs of the anterior pituitary are less well protected by this buffering action in this species than in human. Major differences are also present in the half-life of T_4 in rats (12 h) and human (5 – 9 days), as well as in the serum level of thyroid-stimulating hormone (TSH), which is 25 or more times higher in rats than in human (Capen, 1992). In addition, rats show enhanced thyroid hormone elimination, following conjugation with glucuronide or sulphate (de Herder *et al.*, 1988; Eelkman-Rooda *et al.*, 1989; Visser *et al.*, 1990), with less efficient enterohepatic re-circulation than human (Rutgers et al., 1989). The histology of the resting rat thyroid is similar to that of the stimulated human gland, with small follicles lined by columnar follicular cells. Thus, both physiology and histology indicate that the rat thyroid is more active than the human gland and thyroid hormone turnover in rat is more rapid and subject to significant, short-term variations.

2.3 *Modes of action of thyroid follicular cell tumourigens*.

Genotoxic substances that are able to induce thyroid cancers in rodents appear to have morphological and physiological effects that are different from those of known goiterogens. An example is *N*-bis(2-hydroxypropyl)nitrosamine (DHPN), which induces focal atypical hyperplasia (i.e., arising from single follicles) and, like *N*-nitrosomethylurea, appears to have no effect on the hypothalamic-pituitary-thyroid axis. These observations contrast with the diffuse follicular hyperplasia induced by goiterogens, whose action is primarily at the level of regulation within the thyroid itself, or in the pituitary, liver or other tissues (Kawaoi *et al.,* 1991). Procedures and biochemical perturbations that can have an influence on thyroid function, regulation and histology are listed below.

Thyroid gland
1. Partial thyroidectomy
2. Iodide deficiency
3. Inhibition of iodide pump
4. Inhibition of thyroid peroxidase (TPO)
5. Toxicity to follicular cells
6. Inhibition of thyroid hormone release
Peripheral tissues
7. Inhibition of 5′-monodeiodinase
Liver
8 Enhanced thyroid hormone conjugation and excretion
Pituitary gland
9. Transplantation of TSH-secreting tumour

Mechanistic studies have been pursued only to a modest extent with propylenethiourea, but this is close analogue of ethylenethiourea, which has been well studied, allowing at least cautious extrapolations to be made between these pesticide metabolites. Not all genotoxicity tests with ethylenethiourea have given null results, but it is clear that this activity is not a significant mode of toxicological action, which includes the induction of thyroid follicular tumours in male and female mice and rats and liver and posterior pituitary tumours in mice (WHO, 1993). Genotoxicity tests with propylenethiourea have been less numerous than for ethylenethiourea, but they also point towards genotoxicity not being a significant mode of action. A table of comparisons of toxicological end-points relevant to this discussion is presented below.

Characteristic	Ethylene thiourea[10]	Propylene thiourea[11]
Thyroid: Cellular hypertrophy	Yes	Yes
Hyperplasia	Yes	Yes
Weight increase	Yes	Yes
T4 and T3 decrease	Yes	
TSH increase	Yes	
Liver: Weight increases	Yes (rat & mouse)	Yes (mouse)
Genotoxicity	No	No

Although, in the case of propylenethiourea, there are data gaps (the lack of measurements of plasma hormones) and liver weight increases were observed only in mice, these data are consistent with the hypothesis that propylenethiourea and ethylenethiourea have similar toxicological properties in rats.

A critical action of ethylenethiourea is the reversible inhibition of TPO. In addition, it reduces the uptake of iodide. It therefore has at least two modes of action that would influence the hypothalamic-pituitary-thyroid axis, producing the observed increase in plasma TSH levels. It is important to recognise that the effect of ethylenethiourea on thyroid hormone homeostasis in rats occurs at doses that span the range inducing thyroid tumours in this species.

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[10] WHO, 1993.
[11] Draft assessment report of propineb.