Ethylene thiourea

Health-based recommended occupational exposure limit

Aanbiedingsbrief

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Report of the Dutch Expert Committee on Occupational Standards,
a committee of the Health Council of the Netherlands

to

the Minister and State Secretary of Social Affairs and Employment

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Samenvatting

1 Vraagstelling

Op verzoek van de minister van Sociale Zaken en Werkgelegenheid beveelt de Gezondheidsraad gezondheidskundige advieswaarden aan voor beroepsmatige blootstelling aan toxische stoffen in lucht op de werkplek. Deze aanbevelingen worden opgesteld door de Commissie WGD van de Raad, de opvolgster van de Werkgroep van Deskundigen. Zij vormen de eerste stap in een drietrapsprocedure die moet leiden tot de wettelijke grenswaarden (MAC-waarden).

In het voorliggende rapport bespreekt de commissie de gevolgen van blootstelling aan ethyleenthioureum (ETU) en beveelt zij een gezondheidskundige advieswaarde aan. De conclusies van de commissie zijn gebaseerd op wetenschappelijke publicaties verkregen uit gegevensbestanden van vóór augustus 1995.

2 Fysische en chemische eigenschappen

Bij kamertemperatuur is ETU een reukloos, wit tot lichtgroen kristallijn poeder. Het is in water een tamelijk stabiele verbinding maar wordt gemakkelijk geoxideerd tot ethyleen-ureum. Fijn verstoven in lucht is ETU explosief; de verbinding is ook brandbaar. ETU wordt onder meer gebruikt als intermediair van anti-oxidantia en bij de productie van kunstharsen.

3 Monitoring

De standaardmethode voor de bepaling van ETU-concentraties in de lucht op de werkplek is beschreven in het NIOSH Manual of Analytical Methods. Deze methode berust op spectofotometrie bij een golflengte van 590 nm. De detectielimiet bedraagt 0,0075 mg/m^3 voor een monster van 100 liter bij 0,01 absorptie, bij gebruikmaking van cellen met een optische welglengte van 5 cm.

4 Huidige grenswaarden

Nederland, Duitsland, het Verenigd Koninkrijk en de Verenigde Staten kennen geen grenswaarde voor beroepsmatige blootstelling.

5 Toxicokinetiek

ETU wordt geabsorbeerd in het maagdarmkanaal, de huid en — naar alle waarschijnlijkheid — ook in de luchtwegen. Ter zake zijn geen kwantitatieve gegevens beschikbaar. De stof wordt beperkt in het lichaam verspreid en stapelt zich dan op in de schildklier. Het patroon van biotransformatie verschilt per dier soort. Uitscheiding vindt voornamelijk plaats via de urine (tot 90%), maar in geringe mate ook via de feces en uitademing. ETU en zijn metabolieten hebben een halfwaardetijd van ongeveer 28 uur bij apen, 9 tot 10 uur bij ratten en 5 uur bij mensen. Biologische monitoring is mogelijk aan de hand van concentratiebepalingen in urine. Bij mensen wordt naar schatting in 100 uur de helft van ingenomen ETU via de nieren uitgescheiden.

6 Effecten

ETU is licht irriterend voor de huid. Uitkomsten van onderzoek naar acute toxiciteit geven aanwijzingen dat ratten gevoeliger zijn dan muizen. Blijkens de resultaten naar toxiciteit op korte termijn is de schildklier het meest beïnvloede orgaan. Bij ratten is een reductie gevonden van afscheiding van tri-jodothyronine (T3) en van thyroxine (T4), even als verhoogde afscheiding van TSH (schildklierstimulerend hormoon). Er zijn tussen ratten en muizen kwalitatieve verschillen met betrekking tot het vermogen van de lever om xenobiotische stoffen te metaboliseren. ETU kan ultrastructurele veranderingen geven in de proximale tubuli van nieren van ratten. Ook kan ETU in ratten afwijkingen in het perifere zenuwstelsel teweegbrengen.

Bij ratten veroorzaakt ETU kanker van de schildklier, bij muizen adenomen en carcinomen van de lever. Maar ETU bleek bij hamsters geen kanker te veroorzaken en

het is niet mutageen. De commissie is van mening dat de carcinogeniteit van ETU bij knaagdieren geen betekenis heeft voor de beoordeling van de risico's van beroepsmatige blootstelling. De bij knaagdieren waargenomen verschijnselen blijken verband te houden met karakteristieke facetten van de fysiologie van de schildklier.

ETU is teratogeen bij ratten. De meest bedreigde organen zijn hier het centraal zenuwstelsel, de nieren, de urineleider en het bot.

Er zijn maar weinig gegevens met betrekking tot de mens. In een dwarsdoorsnede-onderzoek is gebleken dat beroepsmatige blootstelling aan ETU leidde tot afwijkingen in het functioneren van de schildklier, zich onder meer uitend in verlaagde thyroxine-serumspiegels. Er is geen doorslaggevend bewijs waaruit zou blijken dat ETU bij de mens allergische contactdermatitis veroorzaakt.

7 Evaluatie en advieswaarde

De commissie vindt dat de gezondheidskundige advieswaarde voor beroepsmatige blootstelling aan ETU gebaseerd moet worden op de uitkomsten van het dwarsdoorsnede- en followuponderzoek bij mensen die beroepsmatige blootstelling ondervinden. Blijkens die uitkomsten leidt blootstelling aan 0,120 mg ETU per kubieke meter lucht tot een significante verlaging van de thyroxine (T4)-concentraties in het serum. De commissie vat deze blootstellingsconcentratie op als de laagste concentratie waarbij een ongunstig effect is waargenomen (LOAEL).

Met hantering van een veiligheidsfactor van 5 komt de commissie tot een advieswaarde voor beroepsmatige blootstelling van: 0,024 mg ETU per kubieke meter lucht, gemiddeld over een werkdag van 8 uur.

Executive summary

1 Scope

At the request of the Minister of Social Affairs and Employment, the Health Council of the Netherlands recommends health-based occupational exposure limits for the concentrations of toxic substances in air at the workplace. These recommendations are made by the Council's Dutch Expert Committee on Occupational Standards (DECOS). They constitute the first step in a three-step procedure that leads to legally binding limit values.

In the present report, the committee discusses the consequences of occupational exposure to ethylene thiourea (ETU) and recommends a health-based occupational exposure limit. The committee's conclusions are based on scientific publications prior to 1995.

2 Physical and chemical properties

ETU is an odourless, white to light green crystalline powder at room temperature. ETU is a fairly stable compound in terms of hydrolitic activity, but is easily oxidized to ethylene urea (EU). ETU is explosive when finely dispersed in air; the compound is flammable. ETU is amongst others used as an intermediate for antioxidants and in the manufacture of synthetic resins.

3 Monitoring

The accepted method of determining atmospheric ETU concentrations at the workplace is described in the NIOSH Manual of Analytical Methods; it is based on use of a spectrophotometer at 590 nm. The detection limit is 0.0075 mg/m^3 in a hundred-litre sample for 0.01 absorbance, using five-centimetre optical path length cells.

4 Current limits

No occupational exposure limits have been set in the Netherlands, the United States, Germany or the United Kingdom.

5 Toxicokinetics

ETU is absorbed by the gastrointestinal tract, the skin and most probably also through the respiratory tract. No quantitative data on uptake through these organs is available. ETU is limited distributed throughout the body and accumulates in the thyroid gland. There are species differences in the pattern of biotransformation. ETU is excreted primarily in the urine (up to 90%), but small quantities are excreted in the faeces and by exhalation. ETU and its metabolites have a half-life of about twenty-eight hours in monkeys, nine to ten hours in rats and five hours in mice. Biological monitoring has been performed by determining ETU concentrations in the urine. In humans, the estimated half-life for elimination of ETU through the kidneys is about one hundred hours.

6 Effects

ETU is slightly irritating to the skin. Acute toxicity studies indicated that rats are more susceptible to ETU than mice. Short-term toxicity studies demonstrated that the thyroid gland is the most affected organ. Reduced T_3* and T_4** secretion and increased TSH (thyroid-stimulating hormone) secretion are found in rats. There are also qualitative differences between rats and mice in terms of their hepatic xenobiotic metabolizing systems. ETU may induce ultrastructural changes in the proximal tubuli of the rat kidneys. The compound may also cause aberrations of the peripheral nervous system of rats.

ETU is carcinogenic to rats, inducing thyroid cancer. In mice, it induces liver adenomas and carcinomas. However, ETU has not been found to be carcinogenic to

* T_3 = Triiodothyronine
** T_4 = Thyroxine

hamsters and it is not mutagenic. The committee endorses the European Union's conclusion that the carcinogenicity of ETU to rodents is not relevant for assessing risks of human occupational exposure. The effects observed appear to be related to characteristics of rodent thyroid gland physiology.

ETU is teratogenic in rats. The foetal organs which are most affected are the central nervous system, the kidneys, the ureter and the skeleton.

Limited human data are available. From a cross-sectional study, it has been demonstrated that occupational exposure to ETU induces aberrations in the functioning of the thyroid gland, e.g. reduced thyroxine levels in the serum. There is no conclusive human evidence that ETU causes allergic contact dermatitis.

7 Hazard assessment and recommended exposure limit

The committee is of the opinion that a health-based recommended occupational exposure limit for ETU should be based on the results of the human cross-sectional and follow-up study of workers occupationally exposed to ETU. From this study the committee concludes that after exposure to an ETU concentration of 0.120 mg/m^3, the T_4 levels in serum are significantly reduced.

Taking this concentration as the lowest observed adverse effect level (LOAEL) and applying a safety factor of 5, the committee recommends an health based occupational exposure limit for ETU of 0.024 mg/m^3 as an 8-hour time weighted average concentration.

Chapter 1

Scope

1.1 Background

In the Netherlands occupational exposure limits for chemical substances are set using a three-step procedure. In the first step a scientific evaluation of the data on the toxicity of the substance is made by the Dutch Expert Committee on Occupational Standards (DECOS), a committee of the Health Council of the Netherlands, on request of the minister of Social Affairs and Employment (Annex A). This evaluation should lead to a health based recommended exposure limit for the concentration in air of the substance. Such an exposure limit cannot be derived if sufficient data are not available or if the toxic action cannot be evaluated using a threshold model. In the latter case an exposure-response relationship is recommended for use in regulatory standard setting.

In the next phase of the three-step procedure the Social and Economic Council advises the minister on the feasibility of using the health based value as a regulatory Occupational Exposure Limit (OEL) or recommends a different OEL. In the final step of the procedure, the Minister of Social Affairs and Employment sets the official Occupational Exposure Limit.

1.2 Committee and method of work

The present document contains the assessment of DECOS, hereafter called the committee, of the health hazard of ethylene thiourea (ETU). The members of the committee are listed in Annex B.

The first draft of this report was prepared by dr AAE Wibowo, from the Coronel Laboratory at the University of Amsterdam, by contract with the Ministry of Social Affairs and Employment.

In 1996 the President of the Health Council released a draft of the report for public review. The individuals and organisations that commented on the draft are listed in annex C. The committee has taken these comments into account in deciding on the final version of the report.

1.3 Data

Literature searches were conducted in the on-line databases Toxline and Medline starting from 1966 up to 1995. In the compilation of this report, extensive use was made of the following review papers:

- Frakes, R.A. (1988). Drinking water guideline for ethylene thiourea, a metabolite of ethylene bisdithiocarbamate fungicides. Reg. Toxicol. Pharmacol. 8, 207-218.
- Khera, K.S. (1987). Ethylene thiourea: a review of teratogenicity and distribution studies and an assessment of reproduction risk. CRC Crit. Rev. Toxicol. 18, 129-139.
- Lentza-Rizos, Ch. (1990). Ethylene thiourea (ETU) in relation to use of ethylenebisdithiocarbamate (EBDC) fungicides. Rev. Environ. Contam. Toxicol. 115, 1-37.
- World Health Organization (1988). Dithiocarbamate pesticides, ethylene thiourea, and propylene thiourea: a general introduction. Environmental Health Criteria 78, 140 pp, Geneva.
- NTP Technical report on the perinatal toxicology and carcinogenesis studies of ethylene thiourea in F344/N rats and $B_6C_3F_1$ mice. NTP Toxicology Program TR 388. March 1992, US Dept. of Health and Human Services.

Before finalising the document the committee performed an additional literature search in Medline and Toxline for the period 1995-1998. The results of this search were no reason for the committee to adjust the recommendations.

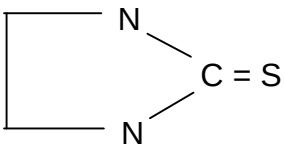
Chapter 2

Identity, physical and chemical properties, monitoring

2.1 Identity

2.1.1 Structure

The chemical structure of ethylene thiourea (ETU) is:



Formula: $C_3H_6N_2S$

2.1.2 Chemical names and synonyms, register numbers

Ethylene thiourea	Imidazoline-2-thiol
ETU	N,N'-ethylene thiourea
2-imidazolidinethione	1,3-ethylene thiourea
2-mercaptoimidazoline	2-imidazoline-2-thiol
CAS reg. number:	96-45-7
UN no.:	2811

RTECS no.:	NI 96250
EINECS:	02-506-9
EEC:	613-039-009

2.2 Physical and chemical properties

Ethylene thiourea is an odourless, white to light green crystalline powder. Its physical properties are as follows:

Relative molecular mass:	102.15
Melting point (°C):	203-204
Relative density (water = 1):	1.4
Solubility in water, g/100ml at 30°C:	2.0
Solubility in ethanol:	moderate
Solubility in chloroform:	nearly insoluble
Log P octanol/water:	-0.7

ETU is a fairly stable compound in terms of hydrolytic reactivity, but it is easily oxidized to ethylene urea, primarily in biological systems and by photolytic reaction. As a powder, ETU is explosive when finely dispersed in air; the compound is flammable.

2.3 Analytical methods

2.3.1 Environmental monitoring

The NIOSH Manual of Analytical Methods (NIO78) describes an analytical method for the environmental monitoring of airborne occupational exposure to ETU. The following synopsis of the method is given:

ETU is collected from air on a PVC or cellulose ester membrane filter. The filter is extracted with distilled water. Pentacyanoamine ferrate reagent is added to the extract to form a coloured coordination complex. The absorbency of the solution is measured spectrophotometrically at 590 nm, and the concentration of ETU is determined from a calibration curve.

The working range of this method is 0.03-1.5 mg/m^3 in a hundred-litre sample. The entire linear working range has not been determined. The sensitivity is 0.006 absorbancy unit/g as determined from the slope of several calibration curves. The detection limit is 0.75 µg/sample or 0.0075 mg/m^3 in a hundred-litre sample for 0.01 absorbency, using five-centimetre optical path length cells.

Other analytical methods for environmental monitoring have been reported since the detection of ETU residues in ethylene bisdithiocarbamate (EBDC) fungicides, used for crop treatment. ETU may contaminate plants, soil or water. Residue analysis consists of sampling the contaminated material, extracting the pesticide residue, identifying and quantifying the residue.

The main methods of analysis described by the WHO (WHO88) were:

- gas liquid chromatography
- thin-layer chromatography
- polarography
- radioisotope dilution
- HPLC.

2.3.2 *Biological monitoring*

The most widely established method of biological monitoring involves analyzing urine samples from occupationally exposed workers to ETU. This analytical technique was described by Kurttio and Savolainen (Kur90) and Kurttio *et al*. (Kur88) and was summarized as follows:

Urine samples are evaporated to dryness or lyophilized, then methanol and silica gel are added. Methanol is evaporated, the sample is transferred to a methanol-washed aluminum oxide column, and diluted with methanol in dichloromethane. The eluate is evaporated to dryness and the sample is dissolved in water and filtered. Filters undergo ultrasonic treatment in water. ETU is then determined by reversed-phase high-performance liquid chromatography with detection at 230 nm. The detection limit for ETU is 0.1 ng per injection and linear response is found for the range of 0.3 to 110 ng; the detection limit in urine is 0.2 µg/l ETU. Recovery from spiked filters (7 µg ETU/filter) varied from 79 to 94%. The methods are sensitive enough for application at the work place. It is pointed out that other methods of exposure should also be taken into consideration in the strategy of monitoring, e.g. exposure to EBDCs (ethylene bisdithiocarbamate) in which ETU also exists as a breakdown product.

It should be noted that ETU is not very stable in either water or urine. Thus, samples should be analyzed as soon as possible after collection.

Chapter 3

Sources of exposure

3.1 Natural occurrence

Ethylene thiourea does not occur naturally.

3.2 Man-made sources

Ethylene thiourea is one of the principal degradation products of the metal salts of ethylene bisdithiocarbamic acid, which are widely used as agricultural fungicides. Although most studies have been performed on the manganese salt (maneb), the sodium salt (nabam) and the zinc salt (zineb), it was found that ETU was present in twenty-eight different commercial EBDC (ethylene bisdithiocarbamate) products (Int74). The amount of ETU present in such products varies between samples and is dependent on the duration between manufacture and use and the storage conditions, especially temperature and moisture (WHO88).

ETU is one of the principal residues found in plants and in the environment following agricultural use of EBDCs. It is also a metabolite formed when EBDCs are ingested by animals and man (see section 6.3).

3.2.1 Production

Although synthesis of ETU was already described in 1872, commercial production was not reported in the US until 1951 (Int74). The substance can be synthesized by reaction

of ethylene diamine with carbon disulphide, followed by refluxing with hydrochloric acid to obtain ring closure. Whether this method is used in commercial production is not known. No data are available on the commercial production of ETU in the Netherlands or other European countries.

3.2.2 Uses

ETU is widely used as an accelerator in neoprene rubber production and in the curing of polyacrylate rubber. It is also used as an intermediate for antioxidants and in the manufacture of synthetic resins. US production in 1980 was probably greater than one million pounds; no current data is available (NTP92).

The various types of neoprene rubber are used almost exclusively in industrial applications, e.g. industrial and mechanical goods, automotive products, wire and cable, construction, adhesives and miscellaneous applications (Int74).

According to the 1994 edition of the Netherlands' National Statistical Yearbook, Dutch national output of synthetic rubber (including latex) was 235 million kilograms in 1985, 236 million kilograms in 1990 and 223 million kilograms in 1991. No breakdown was given, so levels of neoprene and polyacrylate rubber production are not known.

Chapter 4

Environmental levels and human exposure

4.1 Environmental levels

ETU enters the environment primarily as a degradation product of widely used EBDC fungicides. It is also present as a small-volume impurity in these pesticides. The amount of ETU in commercially produced EBDCs has been shown to increase as temperature and humidity increase.

The residue levels of ETU are generally below 0.1 mg per kilogram of product following treatment (with different formulations) at the maximum recommended EBDC levels (WHO88).

4.2 Human exposure

4.2.1 General population

USEPA has estimated that the dietary exposure to ETU of the general population of the USA is not more than 0.004 mg per kilogram body weight per day (WHO88). This maximum exposure was calculated on the assumption that residues are present at the tolerance level and that all of the EBDC residue is quantitatively converted to ETU.

Using actual residue data and experimentally derived conversion factors, the US EPA estimated the dietary intake of ETU to be 0.0002 mg per kilogram body weight per day.

On the other hand, Khera (Khe87) cited a FAO report mentioning that actual daily intakes of 0.001 to 0.007 mg per kilogram body weight for the general population and 0.16 mg per kilogram body weight per exposure for fungicide sprayers had been estimated. These levels were comparable to the temporary human acceptable daily dose of 0.005 mg per kilogram.

The WHO (WHO88) cited a market-basket study of five hundred samples of thirty-four foods, plus twenty-six samples of drinking water. From this Polish data, exposure to ETU was estimated to be 0.00001 to 0.001 mg per kilogram body weight per day.

4.2.2 *Working population*

Three groups of workers are expected to be exposed to ETU: (I) agricultural workers and farmers who are using EBDCs to protect crops, (II) workers engaged in the production of synthetic rubber for which ETU is used as a curing accelerator or vulcanizing agent, (III) ETU production workers and dithiocarbamate production workers.

Agricultural workers

Savolainen *et al*. (Sav89) performed an environmental monitoring study of potato field and pine nursery workers in Finland. Air samples were collected from the breathing zones of the workers throughout the working period and while the pesticide (maneb) was being weighed. The average ETU concentrations in the breathing zones were 0.14 and 0.60 $\mu g/m^3$ for potato field and pine nursery workers respectively. During weighing, the corresponding airborne ETU levels were 0.87 and 1.81 $\mu g/m^3$.

Kurttio *et al*. (Kur90A) performed a study at 14 potato farms in Finland. Air samples were collected from the breathing zones of farmers and from the cabins of their tractors. The airborne concentrations of ETU ranged between 0.004 and 3.3 $\mu g/m^3$ in the breathing zones and between 0.006 and 0.8 $\mu g/m^3$ in the tractor cabins. The authors estimated that the occupational exposures to ETU of these workers was only 3.5% of the acceptable daily intake (ADI = 2 µg/kg/day).

Synthetic rubber production workers

Industrial workers engaged in the manufacture of rubber products for which ETU is used as an accelerator or as vulcanizing agent can be exposed to ETU. A survey of the use of additive chemicals in thirty-eight rubber manufacturing establishments in Sweden, together employing around 14 000 production workers, revealed that about five hundred

substances or products were used in quantities ranging from tenths of a kilogram to some 1 000 tons per year (Hol80).

Very little information is available on the exposure levels to ETU in such industries. Smith (Smi84) reported on a plant at which ETU powder was mixed into an ethylene propylenediene monomer (EPDM) rubber, and the mixture was rolled into sheets. Originally the powder was supplied in paper sacks that were slit open and tipped into a Banbury mixer. This was a dusty operation and in 1980 ETU levels of between 120 and 160 $\mu g/m^3$ were recorded on personal samplers.

ETU production workers

ETU is produced by reacting ethylene diamine with carbon disulphide in a closed reactor. This is followed by various processes to produce a dust-suppressed powder that is bagged off under local ventilation. Some operations are very dusty, and the operators which were exposed to high levels wear respirators; the greatest exposure was believed to occur in the bagging process. Samples of background atmosphere taken from various parts of an ETU manufacturing plant in England showed background ETU levels in the range of 10 to 240 $\mu g/m^3$. Samples from personal monitoring showed ETU levels of up to 330 $\mu g/m^3$ (Smi84).

Occupational exposure to ETU may also occur in workers engaged in producing commercial formulations containing active ingredients of the compounds (Apr98).

Chapter 5

Toxicokinetics

5.1 Absorption

From various animal studies in which ETU has been administered in food or drinking water, or by gastric intubation, it can be concluded that the compound is absorbed rapidly through *the gastrointestinal tract*. According to the WHO (WHO88), ETU appeared in the blood of rats administered an oral dose of 100 mg ^{14}C-ETU per kilogram body weight after five minutes. No quantitative human data is available, but it is assumed that absorption via the gastrointestinal tract is also rapid in humans.

There are indications that ETU is also absorbed through *the skin*. Experimental data showed the induction of embryotoxicity in rats after cutaneous application of ETU (Stu77). After the hair had been removed, the compound was applied by syringe to the intact skin over the thoracic vertebral area. The amount applied was less than 0.5 ml. If a large volume was required, it was applied slowly to allow time for skin penetration. The dose was expressed in milligrams per kilogram body weight and as a fraction of the skin absorption approximate lethal dose (ALD) for pregnant females of the species. The ALD for ETU in rats was found to be 2 250 mg per kilogram body weight (in DMSO). It was determined that ETU is a potent teratogen in rats. For further specification of the effects, see section 6.1.5.

Human data also suggests that ETU may be absorbed through the skin after skin contact. Allergic contact dermatitis induced by ETU has been reported (Bru83; Rud76), but there is no quantitative data.

No data is available to indicate whether ETU can be absorbed via *the lungs* following inhalatory exposure. However, given its physical characteristics, such as its solubility in water, it is probable that ETU passes easily through the lung membranes. Moreover, studies on workers occupationally exposed to ETU showed ETU in their urine samples (Kur90; Kur90B). See also section 2.3.2. on biological monitoring.

5.2 Distribution

ETU is rapidly adsorbed , metabolized and excreted in mammals. Up to 90% is eliminated via the urine. After absorption, ETU is distributed throughout the body and relatively accumulates in the thyroid gland, irrespective of the way of exposure (NTP92). Khera (Khe87A) reported that ETU administered to male rats in a single oral dose of 20 mg/kg was found uniformly distributed in the liver, kidney, heart and muscle with higher concentrations in the thyroid. In another study, involving the administration of ^{14}C-ETU to pregnant rats, radioactivity was uniformly dispersed between the plasma and erythrocytes of the mother, and the radiolabel binding of erythrocytes was shown to be reversible. In the embryo, radioactivity was dispersed uniformly with no evidence of binding to DNA, RNA or protein. Allen *et al*. (All78) studied the distribution of radio-labelled ETU in monkeys and rats. The animals were given the compound by gastric intubation. The authors found that tissue distribution accounted for 21-28% of the administered ^{14}C in the monkeys, while less than 1% was located in the fat tissues. The skin and muscles contained the largest amount of radioactivity in both species.

5.3 Biotransformation and excretion

It has been demonstrated that ETU degradation leads to traces of ethylene urea (EU) and other metabolites in the urine and that ^{14}C-carbon dioxide is exhaled after administration of labelled ETU to mammals (WHO88). Kato *et al*. (Kat76) suggested that the metabolites of ETU in rats were produced primarily by fragmentation of the imidazolidine ring and decarboxylation. Ruddick *et al*. (Rud76), however, concluded that ETU metabolism in rats does not appear to result in any release of ^{14}C into the general metabolic pool. According to the WHO (WHO88), in mice ETU is metabolized into EU and other unknown metabolites, while in rats it is metabolized into S-methyl-ETU and EU. No data is available on the metabolism of ETU in humans.

There seems to be a difference between rats and mice in the effect of ETU on some liver enzyme activities. A comparative study was performed by Lewerenz and Pass (Lew84) in male rats given doses of 50 and 75 mg/kg bw and in mice given doses of 50 to 1,000 mg/kg bw for three days. The authors demonstrated that twenty-four hours after treatment the activity of aminopyrine N-dimethylase in rats was reduced to levels

between 60 and 70% of controls. Decreased aniline hydroxylase activity and cytochrome P-450 content were observed on the third day after exposure. In mice, aminopyrine N-dimethylase was unaffected after treatment with ETU and an increase of cytochrome P-450 content at all dose levels was observed. The activity of aniline hydroxylase was significantly elevated in the groups of mice given doses of 100 mg/kg bw or more.

The effects on aniline hydroxylase in mice and aminopyrine N-dimethylase in rats were substantiated in the experiment performed by Meneguz and Michalek (Men87). On the other hand ETU did not affect aniline hydroxylase activity in rats. Meneguz and Michalek (Men86) also reported that oral administration of ETU to mice at single doses of 50 to 600 mg/kg caused a dose-dependent increase in hepatic microsomal aniline hydroxylase without affecting aminopyrine N-dimethylase activity or total microsomal cytochrome P-450 content. Pre-treatment with actinomycin D completely prevented the increase of aniline hydroxylase. According to Hui *et al*. (Hui88) the mouse metabolizes ETU preferentially via the flavin-dependent mono-oxygenase (FMO) system.

Rapid metabolism of ETU by mice (compared with rats) might explain why the compound is acute toxic and not teratogenic to mice. The FMO-mediated binding of ETU metabolites to mouse liver proteins may contribute to the chronic hepatotoxicity of the compound in this species (Hui88; NTP92).

Allen *et al*. showed that in Rhesus monkeys, ETU is excreted primarily in the urine (All78). The researchers gave two female Rhesus monkeys ^{14}C-ETU by gastric intubation at a dose of 40 mg per kilogram body weight; the rate of excretion was then followed for forty-eight hours. The ^{14}C was excreted mainly in the urine, with 47 and 64% of the total radioactivity being detected in the monkeys' urine. The faeces contained less than 1.5% of the radioactivity.

The WHO (WHO88) reported that the half-life of ETU and its metabolites was about twenty-eight hours in monkeys, nine to ten hours in rats and five hours in mice.

5.4 Biological monitoring

Exposure to ETU can be biologically monitored by determining the amount of the original compound in the urine.

Kurttio *et al*. (Kur90) evaluated the exposure of workers to ETU and EBDCs at twenty-nine potato farms during pesticide application. Biological monitoring was carried out by measuring the concentrations of ETU in urine for twenty-two days after the end of exposure. ETU is an impurity in EBDCs (ethylenebisdithiocarbamate) and can also a metabolite of EBDCs. The estimated inhaled doses of ETU and EBDCs during the average four-hour application period were 0.07 and 1.8 µg/kg respectively. The average concentration of ETU in the breathing zone was 843 ng/m^3. Only 1 to 10% of ETU on the clothes reached the skin. The creatinine-corrected concentrations of ETU in the urine

were 0.1 to 2.5 µg/mmol creatinine, twenty-four hours after cessation of exposure. The estimated half-life for elimination of ETU through the kidneys was approximately a hundred hours.

Kurttio and Savolainen (Kur90) subsequently carried out a biological and hygienic monitoring study of workers on potato farms and pine nurseries during application of EBDC fungicides and analyzed ETU in the breathing zones and in the urine of workers. It was found that the excretion rate of ETU was 6 to 10 ng per hour during the first 60 hours after cessation of exposure, and it diminished thereafter to 0.2 ng per hour over a twenty-two-day observation period.

The results of this study support the view that EBDCs are metabolized to ETU in the human body, because exposure to ETU alone cannot explain the amount of ETU found in urine. No clear correlation was found between airborne EBDC concentrations and ETU levels in the urine. The authors concluded that airborne EBDC concentrations and ETU levels in the urine "indirectly" reflect the level of exposure to EBDCs or ETU.

5.5 Summary of toxicokinetic data

Ethylene thiourea (ETU) is absorbed through the gastrointestinal tract, the skin and most probably also through the lungs. No quantitative data are available on the uptake of ETU through these organs. After absorption, ETU is limited distributed throughout the body and relatively accumulates in the thyroid gland, irrespective of the way of exposure.

Biotransformation of ETU takes place through degradation into ethylene urea (EU) and other metabolites. One author suggested that these metabolites were produced by fragmentation of the imidazolidine ring and decarboxylation. There are differences between animal species in the pattern of biotransformation; no human data is available. ETU is excreted primarily in the urine (up to 90%). Small quantities are excreted in the faeces and by exhalation. ETU and its metabolites have a half-life of about twenty-eight hours in monkeys, nine to ten hours in rats and five hours in mice.

Biological monitoring has been performed by determining ETU concentrations in the urine. All studies involved agricultural workers monitored during ethylene bisdithiocarbamate (EBDC) pesticide application. The estimated half-life for elimination of ETU through the kidneys was close to a hundred hours. The problem with these studies is that there is no evidence to suggest whether ETU found in the urine is derived from exposure to ETU in the form of impurities in inhaled EBDCs, or whether it has been metabolized from EBDC.

Chapter 6

Effects

6.1 Animal experiments

6.1.1 Irritation, sensitization and other acute effects

The irritating and sensitizing properties of ETU have been studied by Matsushita *et al*. (Mat76). To test irritation, ETU was diluted fivefold or tenfold and then injected subcutaneously into the backs of adult Wistar rats (n=4 per group). Observations were made twenty-four hours after injection. The results showed that ETU induced a slight reaction, in the form of various degrees of hyperaemia. The allergenicity of ETU was studied by performing a maximization test with guinea pigs. Each group consisted of ten animals. Two methods of induction were used: (I) intradermal induction, with a concentration of 5%, and (2) topical induction (dermal), with a concentration of 25%. For the challenge, concentrations of 2 and 0.5% ETU were used. The results were graded from weak (grade I) to extreme (grade V). The allergenicity grades twenty-four hours after challenging were for the 2% ETU challenge grade IV and for the 0.5% ETU challenge grade II. Forty-eight hours after challenge, the grades were both grade I.

NTP (NTP92) reported that the acute oral LD_{50} values for rats, mice and hamsters were 1832, 3000 and greater than 3000 mg per kilogram body weight, respectively. Lewerenz and Plass (Lew84) also reported that rats were more susceptible than mice, when the substance was given in a single dose. They found an acute oral LD_{50} of 4000 mg/kg for mice, and for rats (both sexes) an acute oral LD_{50} of 940 mg/kg was found.

6.1.2 *Short-term toxicity*

Effect on the thyroid gland

Short-term animal experiments indicate that in rats the organ primarily affected by exposure to ETU is the thyroid gland.

Kurtio *et al*. (Kur86) studied the effect of administration of 0 to 300 mg/l ETU in drinking water ad libitum for twenty-eight days on the function and morphology of the thyroid glands of rats. ETU induced a dose-dependent inhibition of T_3 (triiodo-thyronine) and T_4 (thyroxine) secretion, and a tenfold increase in the TSH (thyroid-stimulating hormone). Electron-microscopic examination showed that ETU induced ultrastructural changes in the thyroid, namely an increased number of myelin bodies, dilatation of the rough endoplasmic reticulum and increased vacuolization in the epithelial cells of thyroid follicles. The authors estimated that these changes were associated with daily doses ranging from 10.6 to 23.4 mg ETU per kilogram body weight per day. This means that the NOAEL is below 10.6 mg ETU per kilogram bw per day.

O'Neil and Marshall (ONe84) conducted ninety-day feeding trials with male and female rats fed either 75 or 100 mg ETU per kg feed. At the highest dose, slightly elevated relative thyroid weights were found. The results observed in the males were consistent with hyperstimulation of the thyroid gland (elevated T_3/T_4 ratio and elevated serum TSH) due to a partial inhibition of thyroid hormone synthesis. Females appeared to be euthyroid. In the 75 mg/kg feed trial, serum T_4 was found to be significantly depressed for both male and female rats. However, neither T_3 nor TSH was significantly changed, indicating a LOAEL of 75 mg ETU per kg feed (5.6 mg per kilogram bw per day).

In another ninety-day study, Freudenthal *et al*. (Fre77) gave ETU to groups of rats at levels of 0, 1, 5, 25, 125 or 625 mg ETU per kg feed. Rats which had been given the highest dose showed acute clinical signs of poisoning, which were not found at the other doses. At 125 mg/kg feed, substantial decreases in T_3 and T_4 were found, as well as a marked increase in TSH. On the other hand, for the 25 mg/kg feed group, an increase in T_4 and thyroid hyperplasia were found at day 60, which had returned to normal by the ninetieth day. From this experiment it may be concluded that a NOAEL for rats is about 5 mg ETU per kg feed (0.38 mg per kilogram body weight per day).

NTP (NTP92) and Chhabra *et al*. (Chh92) performed a thirteen-week feeding study on rats and mice of both sexes. The exposure levels of ETU were 0, 60, 125, 250, 500 or 750 mg/kg food for rats and 0, 125, 250, 500, 1000 or 2000 mg/kg food for mice. There were no clinical signs of toxicity in the rats and all rats survived to the end of the study. Chemical-related lesions were observed in the thyroid gland, pituitary gland and

liver of the exposed rats at all exposure levels. Thyroid follicular cell adenoma were found in male rats in the 250 to 750 mg/kg exposure groups, and in female rats in the 500 to 750 mg/kg groups. From this experiment it may be concluded that no tumours occurred below the level of 60 mg/kg (approximately 4.5 mg per kilogram body weight per day), which is the NOAEL for this study. No overt clinical signs of chemical toxicity were found in mice. Only diffuse thyroid follicular cell hyperplasia were found in male and female mice at concentrations of 500 mg/kg and higher. This indicates that the NOAEL for mice is 250 mg/kg food (approximately 50 mg per kilogram body weight per day). On the basis of this information, the committee concludes that rats are much more susceptible to the toxic effects of ETU than mice.

A 120-day feeding study of male rats was performed by Graham and Hansen (Gra72). Groups of rats were fed ETU at rates of 0, 50, 100, 500 and 750 mg/kg food. A significant decrease in body weight was found in the groups given 500 and 750 mg ETU per kg food and a significant increase in relative thyroid weight was found in the groups given 100 to 750 mg ETU per kg food. Twenty-four hours after injection of ^{131}I, thyroidal uptake was significantly decreased in the groups given 100 to 750 mg/kg food at different stages of the study. No difference in histological appearance could be detected between sections of the thyroid glands of rats given 50 mg ETU/kg food and sections of the thyroids of the control animals. It can be concluded that the NOAEL in this experiment was 50 mg ETU per kg food (approximately 3.75 mg per kilogram body weight per day).

To study the mechanism by which ETU affects the thyroid gland, Doerge and Takazawa (Doe90) performed a series of biochemical experiments. They found that ETU inhibits thyroid peroxidase (TPX), the enzyme that catalyses synthesis of thyroid hormones. Inhibition of TPX-catalyzed reactions by ETU occurs only in the presence of iodide ions with concomitant oxidate metabolism to imidazoline and bisulphite ions. The results of their experiments demonstrated the existence of a metabolic mechanism for detoxification of ETU in the thyroid, suggesting that low-level or intermittent exposure to ETU has a minimal effect on thyroid hormone production. These findings substantiated the results of the animal feeding studies which had indicated that an NOAEL existed for the effect of ETU on the thyroid gland.

Conclusions

From these short-term animal exposure studies it can be concluded that in rats the thyroid gland is the organ most affected by ETU. Rats are much more susceptible to ETU than mice. The NOAEL in rats is estimated to be 0.38-3.75 mg per kilogram body weight per day, and the LOAEL is about 5.6 mg per kilogram body weight per day. In

mice, the NOAEL for the thyroid is estimated to be 50 mg per kilogram body weight per day.

Effect on the liver

Meneguz and Michalek (Men86) reported that single oral administration to mice between 50 and 600 mg per kilogram body weight caused a dose-dependent increase in hepatic microsomal aniline hydroxylase (AH) but did not effect the aminopyrine N-dimethylase activity or the total microsomal cytochrome P-450 content. Pre-treatment of mice with actinomycin D completely prevented the increase of AH. However, addition of concentrations up to 1 mM actinomycine *in vitro* to an incubation mixture of ETU, did not cause any changes of enzymatic AH-activity. This data suggests induction of AH by means of novoprotein biosynthesis.

In a follow-up study Meneguz and Michalek (Men87) compared the influence of ETU on the hepatic microsomal system of the mouse with the influence on the same system of the rat. They found that ETU treatment caused a dose-dependent decrease of aminopyrine N-dimethylase (47% at 200 mg per kilogram body weight) in rats, but did not observe comparable effects in mice. On the other hand, ETU did not affect aniline hydroxylase activity in rats, but caused a more than twofold increase in mice.

Differences between rats and mice in terms of the effect of ETU on hepatic monooxygenase were also reported by Lewerenz and Plass (Lew84). Male rats and mice were given oral doses of 50 and 75, or 50, 75, 100, 500 and 1000 mg per kilogram per day, respectively, for three days. In rats, the aminopyrine N-dimethylase activity was reduced to 60-70% of the level observed in control animals, twenty-four hours after treatment. Decreases in AH activity and cytochrome P-450 content were observed on the third day after exposure. In mice, administration of ETU resulted in an increase in cytochrome P-450 at all dose levels. The activity of AH was significantly elevated in groups given doses of 100 mg per kilogram per day or more. In mice, aminopyrine dimethylase was unaffected by administration of ETU.

Moller *et al*. (Mol86) administered ETU to male rats at concentrations of 1, 5, 50 and 500 mg/l in drinking water ad libitum for up to eight months. No changes in liver cell morphology were found at the concentrations up to 50 mg/l, but at 500 mg/l ETU induced alterations in the hepatic cell morphology. These alterations included a dramatic increase in the amount of smooth endoplasmic reticulum (SER) with a concomitant reduction in rough endoplasmic reticulum (RER), and a relocation of microbodies and mitochondria to the periphery of the SER. From this experiment it can be concluded that the NOAEL for the effect of ETU on the liver of rats is 50 mg/l ETU in drinking water (15 mg per kilogram body weight per day).

Conclusions

These experiments revealed qualitative differences between ETU-exposed rats and mice in terms of the response of the hepatic xenobiotic metabolizing system. The stimulatory effect on microsomal enzymes observed in mice indicates that mice are able to metabolize ETU to a greater extent than rats. Short-term exposure of rats indicated an NOAEL of 15 mg per kilogram body weight per day for the effect of ETU on liver morphology.

Effect on the kidneys

The effect of ETU on kidney function and morphology in rats was studied by Kurttio *et al*. (Kur91). Rats were exposed to ETU in drinking water ad libitum for twenty-eight days at concentrations of 0, 100, 200 or 300 mg/l. No permanent effects were found on the urinary sodium or potassium excretion, urine osmolality, urine excretion of uric acid, protein or glucose. A tendency towards an increase in vasopressin urinary excretion was observed. No prominent light microscopical changes were observed in the kidney of rats; however, ETU induced clear ultrastructural changes in the epithelium of renal proximal tubuli, as observed by electron microscopy. At the highest dose level, increases in lysosome numbers and myelin as well as vacuolization and edema were observed in the cytoplasm of the epithelial cells of proximal tubulus. Thus, the NOAEL for the effect of ETU on the kidneys of rats is about 200 mg/l in drinking water; this equates to a dose of 17.6 mg per kilogram body weight per day.

Effect on the nervous system

Savolainen *et al*. (Sav86) studied the effect of ETU on the peripheral and central nervous systems of the rat. Rats were exposed to ETU at concentrations of between 0 and 300 mg/l in drinking water ad libitum for twenty-eight days. The isolated ilea of all rats showed a slightly increased response to 5-hydroxy-tryptamin. ETU also increased the response of ilea to nicotine. ETU had only a marginal decreasing effect on acetylcholinesterase. The present data suggests that cholinergic peripheral nerves are more susceptible to the neurotoxic effects of ETU than the central nervous system. No threshold concentrations can be determined from these experiments.

6.1.3 Long-term toxicity/carcinogenicity

An overview of long-term studies is presented in Table 1. These studies mostly involved rats. As mentioned earlier, both short-term toxicity studies and long-term toxicity studies

revealed differences between species in terms of the organs primarily affected and in terms of susceptibility to ETU-induced aberrations.

In rats, the thyroid gland was found to be the most affected organ, both in short-term and long-term studies. In the latter, a dose-dependent increase in thyroid cancers was found (Ull72; Wei81; Gra73; Gra75; Gak76; Chh92). No thyroid cancers were found by Graham *et al*. (Gra73, Gra75) below doses of 9.38 mg per kilogram per day. Gak *et al*. (Gak76) reported no thyroid cancers below 1.28 mg per kilogram per day and Chhabra *et al*. (Chh92) and NTP (NTP92) found no thyroid cancers below 1.88 mg per kilogram per day.

In mice a different pattern of carcinogenicity occurs. The liver seems to be the organ primarily affected. Increased incidences of adenomas and carcinomas of the liver were found at a dose of 66 mg per kilogram per day for twenty-four months (Chh92). At higher doses (200 mg per kilogram per day), other organs were also affected; thyroid and pituitary tumours were detected. An increased incidence of tumours was not found in hamsters at doses of between 0.60 and 24 mg per kilogram per day for twenty months (Gak76).

Long-term exposure induces not only tumours, but also other aberrations. In a long-term study reported by NTP (NTP92), it was found that non-neoplastic lesions were associated with the administration of ETU. The substance induced follicular cell hyperplasia in rats and mice and follicular cell cytoplasmic vacuolation, centrilobular hepatocellular cytomegaly, and focal hyperplasia of the pars distalis of the pituitary gland in mice. Other effects associated with the administration of ETU included decreased serum levels of T_4 and/or T_3 in rats and increased serum levels of TSH in rats and mice.

Yoshida *et al*. (Yos93) reported a study performed to investigate possible tumour induction mechanisms in mice. In this study, ETU was administered in combination with sodium nitrite. ETU and sodium nitrite were administered once a week for ten weeks in the following combinations (ETU + sodium nitrite in mg per kilogram bw per week): 0 + 0, 100 + 0, 0 + 70, 25 + 17.5, 50 + 35 and 100 + 70. Thereafter the animals were allowed to live without treatment up to eighteen months after first administration. It was found that ETU and sodium nitrite caused earlier development of tumours and/or dose-dependent increases of tumours in the lymphatic tissue, lung, forestomach, Harder's glands and uterus, whereas treatment with either ETU or sodium nitrite alone had no apparent carcinogenic effect. These results indicate that ETU is most probably converted *in vivo* into *N-nitroso-ETU* and that N-nitroso-ETU has a greater carcinogenic potential in mice than ETU alone.

The mechanism of the thyroid follicular cell carcinogenesis in rats has been reviewed by Chhabra *et al*. (Chh92). They suggested that follicular cell thyroid carcinogens

perturb the thyroid hormone balance in animals, and if sustained for a prolonged period, result in increased incidence of thyroid tumours. The authors proposed that dietary iodide deficiency, thyroidectomy and exposure to chemicals toxic to the thyroid glands cause a reduction of thyroid hormones (T_3, T_4) which triggers hypersecretion of thyroid-stimulating hormone by the pituitary glands. If TSH secretion is prolonged, it may result in follicular cell hypertrophy, hyperplasia, adenomas and carcinomas. ETU carcinogenicity in rats is considered to be due to an imbalance of the thyroid-pituitary homeostasis.

6.1.4 *Mutagenicity*

The following summary is provided by NTP (NTP92).

ETU has been tested extensively for genotoxicity in a variety of *in vitro* and *in vivo* systems, and the results, with a few exceptions, are negative. Results of bacterial gene mutation studies with several strains of Escherichia Coli and Salmonella typhimurium were negative, except for isolated positive responses reported with S. typhimurium strains TA1535.

Results from studies of genetic effects in yeast showed some potential for induction of mitotic aneuploidy, gene conversion and DNA damage. No induction of sex-linked recessive lethal mutations was observed in germ cells of male Drosophila melanogaster treated with ETU by feeding or injection.

ETU was tested in mammalian cells *in vitro* for induction of chromosomal aberrations, sister chromatid exchanges (SCE) and unscheduled DNA synthesis; all results were negative. Positive results were reported in a mouse lymphoma assay for induction of trifluorothymidine resistance in L5178Y cells.

In vivo mammalian tests for induction of micronuclei or sister chromatid exchanges in bone marrow cells of mice were negative, as were tests for induction of dominant lethal mutations or sperm abnormalities.

In contrast to the generally negative genotoxicity test results seen with ETU, its nitrosated metabolite, *N-nitroso-ETU*, was positive for all genotoxicity endpoints for which it was tested, both *in vitro* and *in vivo*. It induced gene mutations in S-typhimurium, sister chromatid exchanges in Chinese hamster V79 cells, and chromosomal aberrations, micronuclei and dominant lethal mutations in mice *in vivo*.

The WHO (1988) also concluded that ETU itself is generally not mutagenic, especially not in mammalian test systems. The IARC (Int87A; Int87B) in their assessment also pointed out that most genotoxicity studies were negative. Studies on gene conversion and DNA damage in yeast and on mutation in bacteria gave conflicting results.

However, a recent extensive review by Dearfield (Dea94) reported that ETU has a weak genotoxic potential (eg gene mutations, structural chromosomal abberations). These allegation was contradicted by Elia (Eli95), who suggested that the thyroid tumors

Table 1 Overview of research into the effects of long-term exposure to ETU on laboratory animals.

species	method of admini-stration	ETU dosage mg/kg food	duration of exposure	effect	comments	referenc e
rat (male and female), N=26	diet	175 (13 mg/kg/d)	18 months	6/26 thyroid cancers	no cancer in controls	Ull72
per group		350 (26 mg/kg/d)	18 months	26/26 thyroid cancers	observation period: 6 months	Wei81
rat (male and female)	diet	5 (0.38 mg/kg/d)	24 months	2/75 thyroid cancers	in control group 2/72 thyroid cancers	Gra73; Gra75
N = 69-73 per group		25 (1.9 mg/kg/d)		1/73 thyroid cancers	no thyroid cancer at or below 125 mg/kg food	
		125 (9.38 mg/kg/d)		2/73 thyroid cancers	tumour metastases to the lungs	
		250 (18.75 mg/kg/d)		16/69 thyroid cancers		
		500 (37.50 mg/kg/d)		62/70 thyroid cancers		
rat (male and female)	diet	5 (0.38 mg/kg/d)	24 months		no malignant tumours at 17 mg/kg food	Gak76
		17 (1.28 mg/kg/d)				
N = 40 per group		60 (4.5 mg/kg/d)		significant increase malignant tumours	no significant increase in tumours in controls	
		200 (15 mg/kg/d)		significant increase malignant tumours	thyroid impairment predominant	
rat (male and female)	diet	9-90 mg/kg food	perinatal period + 24 months' observation	no effect on incidence of thyroid cancer	no thyroid tumours at 25 mg ETU/kg food	Chh92
N = 60 per group					or a dose of 1.88 mg/kg/d	NTP92
		9-90 mg/kg food continued with	perinatal period	increased incidence of thyroid follicular adenoma or carcinoma		
		25-250 mg/kg food	24 months			
		83 mg/kg food (6.23 mg/kg/d)	24 months	male: 12/46 thyroid tumours female: 7/44 thyroid tumours		
		250 mg/kg food (18.75 mg/kg/d)	24 months	male: 37/50 thyroid tumours female 30/49 thyroid tumours		
		control	24 months	male 1/49 thyroid tumours female 3/50 thyroid tumours		
mouse (male and female)	diet	33-330 mg/kg	perinatal period + 24 months' observation	not carcinogenic	no threshold can be determined for tumours	
N = 60 per group		33-330 mg/kg continued with	perinatal period	increased incidence of adenomas and carcinomas of the liver, thyroid and pituitary glands		
		100-1000 mg/kg	24 months			
		330 mg/kg (66 mg/kg/d)	24 months	male:32/50 liver tumours female: 44/50 liver tumours		
		1000 mg/kg (200 mg/kg/d)	24 months	increased incidence of liver, thyroid and pituitary tumours		

Table 1 Continued.

species	method of admini-stration	ETU dosage mg/kg food	duration of exposure	effect	comments	referenc e
mouse (male and female) N = 60 per group	oral	100 mg/kg/week, 1x/week	10 weeks observation: 18 months	no increased incidence of tumours	ETU in combination with sodium nitrite caused increased incidence of tumours	Yos93
mouse (2 strains)	oral (stomach tube for 4 weeks, then in diet)	215 mg/kg/d, then in diet 646 mg/kg	18 months	increased incidence of hepatomas in both strains	the dose used is the MTD	Inn69
hamster (male and female) N = 40 per group	diet	5 mg/kg (0.60 mg/kg/d) 17 mg/kg (2.00 mg/kg/d) 60 mg/kg (7.20 mg/kg/d) 200 mg/kg (24 mg/kg/d)	20 months	no increased incidence of tumours		Gak76

in rats and liver tumors in mouse were induced by a non-genotoxic mechanism.

6.1.5 *Effect on reproduction*

An overview of research into the reproduction toxicity of ETU conducted using laboratory animals is provided in Table 2.

From the data in Table 2 it can be concluded that ETU is teratogenic to rats. The organs primarily affected in the foetus seem to be the central nervous system (Che79; Hun86; Khe85; Khe87B; Sai91), the kidneys and ureter (Das88; Hun86; Khe89) and the skeleton (Stu77). The level of exposure, method of administration and the dosing period during pregnancy may help to determine which organs are affected and the type of malformation induced. Most experiments involved administration of ETU by gastric intubation; Thus, ETU was shown to have a positive teratogenic effect on rats. Skin application of ETU also led to the induction of skeletal malformations (Stu77). On the other hand, ETU administered by inhalation did not appear to have any teratogenic effect (Dil77); this data should be treated with caution, since the source of information is an incomplete abstract. For the teratogenic effect on rats, there appears to be an NOAEL between 10 mg per kilogram per day (Che79) and 15 mg per kilogram per day (Sai91), administered during the period of pregnancy.

At the given doses, ETU does not seem to be teratogenic to mice, hamsters or guinea pigs (Che79).

In vitro experiments have been performed to establish why rats are more susceptible to the teratogenic effects of ETU than mice (Das89). Comparably staged rat and mouse embryos (gestation days 10.5 and 8.5, respectively) were exposed to static concentrations of ETU for forty-eight hours. The teratogenic effects of ETU were qualitatively similar in both species, characterized by excessive fluid accumulations in embryonic structures. The most common abnormalities were distended neural tubes, especially in the hindbrain, and clear blisters on the caudal region. At least twice as much ETU was required to produce an abnormality incidence in mice similar to that in rats. It was also found that mouse liver microsome S-9 mix virtually eliminated the formation of abnormalities typical of ETU in both rat and mouse embryos.

Daston *et al*. (Das87) reported that an *in vitro* culture of a rat embryo (gestation day 10), for forty-eight hours exposed to between 40 and 200 µg/ml ETU, showed a dose-related inhibition of growth and differentiation as assessed from crown-rump length, protein and DNA content and somite number, and an increase in the frequency of abnormalities. Tsuchiya *et al*. (Tsu91) reported that malformations in rat embryos (gestation days 11 to 13) occurred at concentrations as low as 30 µg/ml ETU, in the head, tail, limbs and face. Other *in vitro* experiments on rat embryos also showed that the central nervous system is one of the organs most susceptible to teratogenic effects of ETU (Khe87B, Khe89, Das90).

Conclusion

ETU is teratogenic to rats and the overall NOAEL for the teratogenic effect on this species is estimated to be 10 mg per kilogram body weight per day. Rats are much more susceptible to ETU than mice. The foetal organs primarily affected are the central nervous system, the kidney and ureter and the skeletal system. The level of exposure, method of administration and the period of dosing during pregnancy may influence the degree of teratogenicity.

6.2 Observations in man

There are little human data available on the effect of occupational exposure to ETU. Smith (Smi76) investigated retrospectively 699 female workers who had come into contact with rubber containing ETU before 1972. The women in question were employed at departments of a rubber manufacturing industry where the following activities were undertaken: hand and machine trimming, hand cutting, coolant hose

Table 2 Overview of research into the reproduction toxicity of ETU conducted using laboratory animals.

species of aimal	method of admini-stration	ETU dosage mg/kg bw/day	dosing period during pregnancy	effect on the foetus	comments	referenc e
rat, N = 8-31 dams per group	gastric intubation	5, 10, 20, 30, 40 or 80 mg/kg bw /day	days 7-21 40 mg/ kg bw/d: CNS	80 mg/kg bw/d is lethal. defects: Low incidence of digital defects and kyphosis. 20 and 30 mg/kg bw/d: hydrocephalus 5 and 10 mg/kg/d: no defects	the NOAEL is estimated to be 10 mg/kg bw/d	Che79
rat, N = 5-6 dams per group	gastric intubation	20, 40 or 60 mg/kg bw (single) 10 mg/ kg bw in pilot study	day 11	60 mg/kg: hydronephrosis and neonatal deaths. Decreased GFR and plasma clearance	urinary system was the most severely affected organ system. Effects detected even at 10 mg/kg bw.	Das88
rat, N = 16-30 foetuses per group	gastric intubation	60, 120, 240 mg/kg bw (single)	day 11 day 12 or 13 or 14 days 15-18	spinal dysraphism exencephaly meningo encephalocele hydraencephaly hydrocephalus	congenital malformation of the CNS is principal effect. No threshold can be determined	Hun86
rat, N = 20 dams per group	gastric intubation	60, 120, 240 or 360 mg/kg bw (single)	day 11	dose-dependent abdominal wall and neural tume (gross) defect	at 60 mg/kg bw no defects seen	Hun92
rat, N = 4-6 dams per group	gastric intubation	15 or 30 mg/kg bw (single)	day 13	karyorrhexis in the germinal layer of basal lamina of CNS. Necrosis of neuroblasts. Hydrocephalus and microphthalmia	effects detected even seen after single dose of 15 mg/kg bw	Khe85
rat, N = 8 dams per group	gastric intubation	30 or 45 mg/kg bw (single)	day 19	necrosis of neuroblasts. High incidence of hydrocephalus		Khe87B
rat, N = 6 dams per group	gastric intubation	60 or 120 mg/kg bw (single)	day 10	no hydrocephalus, but anomalies in the kidney and ureter	effects dependent on dosing in period of pregnancy	Khe89
		45 or 60 mg/kg (single)	day 12	hydrocephalus and general edema		
rat, N = 16-22 litters per group	gastric intubation	15, 25 or 35 mg/kg bw/day	days 6-20	cranial meningocele and meningorrhea, severe hind limb talipes, delated brain ventricles, hydroureter and skeletal immaturities	most affected foetuses had multiple defects. NOAEL for teratogenic effects estimated to be 15 mg/kg bw/d	Sai91
rat, N = n.k.	inhalation	120 mg/m^3	days 7-14 continuously	increased foetal mortality and decreased foetal weight. No teratogenic effects found	abstract only	

Table 2 Continued.

species of aimal	method of admini-stration	ETU dosage	dosing period during pregnancy	effect on the foetus	comments	referenc e
rat, N = n.k.	inhalation	120 mg/m^3	days 7-14 continuously	increased foetal mortality and decreased foetal weight. No teratogenic effects found	abstract only	Dil77
Rat, N = 5-8 dams per group	skin application (dissolved either in water or in DMSO)	25, 50 or 6000 mg/kg bw/d	days 10-13	skeletal malformation		Stu77
mouse, N = 25 dams per group	gastric intubation	100 or 200 mg/kg bw/day	days 7-16	a dose-related increase in relative liver weight. The only foetal alteration was an increase in supernumerary ribs	no foetal toxicity	Che79
hamster, N = 9-15 dams per group	gastric intubation	25, 50 or 100 mg/kg bw/day	days 5-10	no compound-related effect		Che79
guinea pig, N = 3-6 dams per group	gastric intubation	50 or 100 mg/kg bw/day	days 7-25	no compound-related effect on maternal or foetal parameters studied		Che79

manufacture and packing and despatch. The names and dates of birth (1918 or later) of all women of childbearing age who had left employment at the factory between 1963 and 1971 were obtained. They were compared with birth records from the City of Birmingham, which were coded in the same matter and noted foetal abnormalities. The results did not show a significant number of abnormalities. The numbers are relatively small and no definite conclusions can be drawn. The same study also failed to detect any increase in the incidence of thyroid cancer when a total of 1929 workers were compared with the thyroid cancer list of the Birmingham Cancer Registry 1957 to 1971.

In 1984, Smith (Smi84) performed a cross-sectional study of workers at one ETU production plant and at one rubber production factory in the United Kingdom. The value of this study is somewhat limited because of the small number of workers and the intermittent nature of the working with rubber in 1980 and 1981 (due to economic recession). At the ETU production factory, ETU levels of up to 330 µg/m^3 were found in personal air samples; the environmental monitoring levels were in the range of 10 to 240 µg/m^3. The average length of exposure of these workers was ten years with a range of five to twenty years. At the mixing factory, levels between 120 and 160 µg/m^3 were recorded on personal samplers. The studied population was made up entirely of males, ranging in age from 26 to 62 years at the production factory (N = 23) and 28 to 56 years at the mixing factory (N = 22). A control group (N = 40) matched in age and ethnic

origin was also obtained. This group contained other production workers and managerial staff.

The thyroid function of each worker was determined by measuring the T_4*, TSH and thyroid binding globulin (TBG) in serum. The geometric means of T_4 levels were 80.5 nmol/l for mixers, 96.4 nmol/l for process workers and 105.7 nmol/l for controls (normal reference range is 65 to 155 nmol/l according to Mos90). No exposed workers or controls had a history of thyroid illness or of taking thyroid medication, and no clinical symptoms of thyroid disease were found. The concentrations of T_4 from the mixers were clearly lower than those from the control group. The difference between the controls and the process workers was slight, and was entirely due to one individual having a particularly low level. The difference between the mixers and the process workers lay on the borderline of statistical significance. The TSH levels were within normal limits in all the controls and process workers (range 2.1 to 5.4 nmol/l). In one mixer the levels on two occasions were extremely high (21.8 nmol/l and 17.0 nmol/l); he was investigated further and was found to have premyxoedema. The TBG levels and the T_4/TBG ratios did not differ among the groups and were within normal limits.

Rudzki *et al*. (Rud76) performed patch tests on 200 patients with contact dermatitis in Poland. ETU was diluted in yellow soft paraffin and applied to the skin at a concentration of 2%. Only one person out of the 200 (0.50%) exhibited a positive reaction to ETU. Bruze and Fregert (Bru83) reported a case of allergic contact dermatitis to ETU. The patient was a 53-year-old female. For thirteen years, she had worked in an industry manufacturing products from synthetic and natural rubber. Patch testing showed that the patient exhibited a positive reaction to nickel, cobalt and ETU.

6.3 Summary of effects

De committee summarizes the effects of ETU exposure as follows:

- ETU is slightly irritating to the skin. Acute toxicity studies indicate that rats are more susceptible to ETU than mice.
- Short-term exposure toxicity studies in experimental animals indicate that the thyroid gland is one of the principal organs affected by ETU. Rats appear to be much more susceptible than mice. Reductions in T_3 and T_4 secretion and an increase in TSH secretion are found in rats. The NOAEL for these effects in the rat is estimated to be between 0.38 and 3.75 mg per kilogram body weight per day, and the LOAEL 5.6 mg per kilogram body weight per day. The NOAEL in mice for these effects is estimated to be 50 mg per kilogram body weight per day.

* T_4 = Thyroxine

- Short-term toxicity studies revealed qualitative differences between rats and mice in terms of their hepatic xenobiotic metabolizing systems. It has been observed that mice are able to metabolize ETU to a greater extent than rats. It is estimated that the NOAEL for liver effects in rats is about 15 mg per kilogram body weight per day.
- ETU may induce ultrastructural changes in the proximal tubuli of the kidneys of rats after short-term exposure. The NOAEL for these effects is 17.6 mg per kilogram body weight per day. ETU may also cause aberrations of the peripheral nervous system, but no threshold can be determined, although it is probably high.
- ETU is carcinogenic to rats, inducing thyroid cancer. The incidence of thyroid cancer is dose-dependent. In three different experiments, no tumours were found below doses of, respectively, 1.28, 1.88 and 9.38 mg per kilogram body weight per day. In mice, ETU induces liver adenomas and carcinomas. Even at the lowest dose tested (66 mg per kilogram body weight per day) tumours were induced. No tumours were found in hamsters given doses ranging from 0.60 to 24 mg per kilogram body weight per day.
- Studies using mice showed that ETU and sodium nitrite together caused earlier development of tumours and dose-dependent increases in tumours. The hypothesis is that this was due to the formation of N-nitroso-ETU.
- ETU itself is generally not mutagenic, especially in mammalian test systems.
- ETU is teratogenic in rats. The overall NOAEL for this effect in rats is estimated at 10 mg per kilogram body weight per day administered during pregnancy period. Rats are much more susceptible than mice. The foetal organs primarily affected are the central nervous system, the kidneys and ureter and the skeleton. The level of exposure, method of administration and the period of dosing during pregnancy influence the degree of teratogenicity.
- Very little human data is available. From one cross-sectional study there are indications that occupational exposure to 120-160 $\mu g/m^3$ ETU induces alterations of the thyroid function, e.g. reduction in serum levels of thyroxine.

Chapter 7

Existing guidelines, standards and evaluations

7.1 General population

The joint FAO/WHO Meeting on Pesticide Residues (JMPR) resulted in 1988 in an extended evaluation of available data on the toxicological properties of ETU (Len90). Given that the oral no-effect level for teratogenicity is 15 mg/kg body weight, it was estimated that the Acceptable Daily Intake (ADI) for man was 2 µg per kilogram body weight. The reason for the high safety factor of 7 500 was not explained. The 1986 JMPR indicated that the level proposed should be considered as a temporary ADI (T-ADI) and recommended further toxicological studies.

Additional data submitted to and evaluated by the 1988 JMPR intensified the concerns about the toxic properties of ETU, insofar as the compound appeared to be developmentally toxic and goitrogenic. However, the data were not considered sufficient to warrant a complete re-evaluation of the T-ADI. The validity of the T-ADI was therefore extended and additional toxicological studies were requested.

In 1993, the joint FAO/WHO Meeting on Pesticides Residues in Food and Environment resulted in an extended evaluation of available data on the toxicological properties of ETU. The Acceptable Daily Intake (ADI) was based on a NOAEL of 0.39 mg/kg bw/day in a 13 weeks dog study. A safety factor of 100 was applied. The recommended ADI for humans was 0 to 4 µg/kg bw (Foo93).

7.2 Working population

NIOSH (NIO78) indicated that it would be prudent to handle ETU at the workplace as if it were a human carcinogen and teratogen. Exposure to ETU should be limited to as few employees as possible, while minimizing workplace exposure levels. Access to the area in which the substance is used should be restricted to those employees essential to the process or operation. These recommendations were made pending completion of the NIOSH special occupational hazard review and the development of specific recommended control measures for ETU.

Table 3 Occupational exposure standards in various countries.

country	standards (mg/m^3)	comments	reference
The Netherlands (MAC)	-		SZW98
United States of America			
ACGIH (TLV)	-		ACGIH97
OSHA (PEL)	-		
NIOSH (REL)	-		
Germany			
DFG (MAK)	-	classified as Group III B	DFG97
United Kingdom			
HSE (OES)	-		HSE93
Sweden (OEL)	-		SND90
Finland (OEL)	0.2	TWA-8 h	Työ96

Chapter 8

Hazard assessment

8.1 Assessment of health risk

The general public may be exposed to ETU primarily as a result of consuming fruit or vegetables sprayed with ethylene bisdithiocarbamate fungicides; these fungicides contain ETU as degradation product. Occupational exposure occurs during the manufacture of rubber products for which ETU was used as an accelerator or vulcanizing agent and during the manufacture, handling and spraying of ethylene bisdithiocarbamate fungicides.

The exposure routes of primary importance at the workplace are the inhalation of ETU containing dust or through contact with the skin; for the latter pathway no quantitative data are available.

Animal data indicate that ETU is carcinogenic as well as teratogenic. Both the target organs and the sensitivity differ from one species to another. In rats, the thyroid gland is the organ primarily affected and ETU exposure leads to a dose-dependent thyroid tumour induction. The NOAEL for thyroid tumours in rats is 1.88 mg per kilogram body weight per day in case of oral exposure via the diet (Chh92). The carcinogenic effects of ETU in rats seem to be based on an epigenetic mechanism, since ETU is not mutagenic in mammalian systems. The NOAEL obtained in short-term toxicity studies in rats is comparable to the one of the chronic studies: 0.38 mg per kilogram body weight per day (Fre77).

NOAELs and LOAELs, found in other studies, are presented in table 4.

Tabel 4 From animal studies, the following threshold levels can be determined.

dose (mg/kg b.w./day)	exposure	threshold	effects determined
0.38	short-term	NOAEL (rat)	thyroid function (Fre77)
1.88	long-term	NOAEL (rat)	thyroid cancer (Chh92)
5.6	short-term	LOAEL (rat)	thyroid function (ONe84)
10	during pregnancy period	LOAEL (rat)	teratogenicity (Che79)
15	short-term	NOAEL (rat)	liver morphology (Mol 86)
17.6	short-term	NOAEL (rat)	proximal tubuli of the kidney (Kur91)
50	short-term	NOAEL (mice)	thyroid function (Chh92)
66	long-term	lowest dose used (mice)[a]	liver cancer (Chh92)

[a] threshold level could not be determined

Whether ETU can induce thyroid tumours in humans is still a matter of debate (McC92, Ali94). In rodents, it is known that prolonged administration of antithyroid drugs may lead to the development of multiple thyroid tumours (Tho91). However, there are marked differences between rodents and primates in (1) thyroid gland physiology, (2) the spontaneous incidence of thyroid gland neoplasia and (3) the susceptibility to neoplasia secondary to simple hypothyroidism. The authors suggested that rat thyroid is vastly more susceptible to secondary carcinogenesis than the human thyroid. The reason for this is that rats lack high-affinity thyroxine-binding globulin, they are more sensitive to thyroid peroxidase inhibition, and they are more susceptible to hepatic enzyme induction leading to increased breakdown of thyroid hormones (Ali94). In contrast, no clear etiologic role has been established for hypothyroidism in human thyroid cancer, even though chronic hypothyroidism has occurred in humans in areas of endemic goiter. Thus, if simple hypothyroidism contributes to thyroid cancer in humans at all, its contribution is small (McC92).

Therefore, the committee concludes that because of the marked species differences between rats and human in thyroid gland physiology and the apparent susceptibility to hypothyroidism, the rodent is an inappropriate model for assessing the thyroid cancer risk to man from chemicals that induce hormone imbalance, and therefore studies in rodents cannot be the basis for the HBR-OEL.

In mice, the liver is the principal organ affected; ETU induces adenomas and carcinomas. However, the committee is of the opinion that induction of liver tumours in mice is, in general, not relevant for the human risk assessment, and therefore cannot be

used a the starting point for the establishment of a HBR-OEL. At much higher dosages, tumours of the thyroid and pituitary glands were found in mice as well.

Although limited human epidemiological data is available, the committee concludes that the thyroid gland is the principal organ affected after occupational exposure to ETU. A cross-sectional and follow-up study performed by Smith (Smi84) demonstrated significantly lower T_4 levels in the serum of a group of mixers occupationally exposed to a concentration of ETU between 120 and 160 $\mu g/m^3$ (based on personal sampling) than in the serum of a control group. The committee concludes that this study should form the basis for the health risk assessment. Although the exposed workers did not show any clinical symptoms of hypothyroidism, the T_4 levels were significantly decreased, and therefore the committee considers the level of 120 $\mu g/m^3$ ETU as the LOAEL found in humans.

A safety factor of five is applied to compensate for the inter-individual variations and for the fact that the HBR-OEL is based on a LOAEL rather than on a NOAEL. Thus, the committee proposes a health based occupational exposure limit for ETU of 0.024 mg/m^3, as a time-weighted eight-hour average.

The committee is of the opinion that with respect to the carcinogenic effects, ETU should not be classified based on the available (animal) data. The effects on rats and mice are not considered to be relevant to man.

8.2 Groups at extra risk

The committee is not aware of groups to be at extra risk for ETU exposure.

8.3 Health-based recommended occupational exposure limit

The committee recommends a health based occupational exposure limit of 0.024 mg/m^3, as an eight-hour time-weighted average concentration.

For the committee,
The Hague, 30 August 1999

dr ASAM van der Burght,	prof. dr GJ Mulder,
scientific secretary	chairman

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Annexes

Annex **A**

Request for advice

In a letter dated October 11, 1993, ref DGA/G/TOS/93/07732A, to, the State Secretary of Welfare, Health and Cultural Affairs, the Minister of Social Affairs and Employment wrote:

Some time ago a policy proposal has been formulated, as part of the simplification of the governmental advisory structure, to improve the integration of the development of recommendations for health based occupation standards and the development of comparable standards for the general population. A consequence of this policy proposal is the initiative to transfer the activities of the Dutch Expert Committee on Occupational Standards (DECOS) to the Health Council. DECOS has been established by ministerial decree of 2 June 1976. Its primary task is to recommend health based occupational exposure limits as the first step in the process of establishing Maximal Accepted Concentrations (MAC-values) for substances at the work place.

In an addendum, the Minister detailed his request to the Health Council as follows:

The Health Council should advice the Minister of Social Affairs and Employment on the hygienic aspects of his policy to protect workers against exposure to chemicals. Primarily, the Council should report on health based recommended exposure limits as a basis for (regulatory) exposure limits for air quality at the work place. This implies:

- A scientific evaluation of all relevant data on the health effects of exposure to substances using a criteria-document that will be made available to the Health Council as part of a specific request for advice. If possible this evaluation should lead to a health based recommended exposure limit, or, in

the case of genotoxic carcinogens, a ‘exposure versus tumour incidence range’ and a calculated concentration in air corresponding with reference tumour incidences of 10^{-4} and 10^{-6} per year.

- The evaluation of documents review the basis of occupational exposure limits that have been recently established in other countries.
- Recommending classifications for substances as part of the occupational hygiene policy of the government. In any case this regards the list of carcinogenic substances, for which the classification criteria of the Directive of the European Communities of 27 June 1967 (67/548/EEG) are used.
- Reporting on other subjects that will be specified at a later date.

In his letter of 14 December 1993, ref U 6102/WP/MK/459, to the Minister of Social Affairs and Employment the President of the Health Council agreed to establish DECOS as a Committee of the Health Council. The membership of the Committee is given in annex B.

Annex **B**

The committee

- GJ Mulder, *chairman*
  professor of toxicology; Leiden University, Leiden
- RB Beems
  toxicologic pathologist; National Institute of Public Health and the Environment, Bilthoven
- JJAM Brokamp, *advisor*
  Social and Economic Council, Den Haag
- VJ Feron,
  professor of toxicology; TNO Nutrition and Food Research Institute, Zeist
- DJJ Heederik
  Epidemiologist; Agricultural University, Wageningen
- PTh Henderson
  professor of toxicology; Maastricht University, Maastricht
- LCMP Hontelez, *advisor*
  Ministry of Social Affairs and Employment, Den Haag
- G de Jong
  occupational physician; Shell International Petroleum Maatschappij, Den Haag.
- J Molier-Bloot
  occupational physician; BMD Akers bv, Amsterdam
- H Roelfzema, *advisor*
  Ministry of Health, Welfare and Sports, Den Haag

- T Smid
  occupational hygienist; KLM Health Safety & Environment, Schiphol and professor of working conditions, Free University, Amsterdam
- GMH Swaen
  epidemiologist; Maastricht University, Maastricht
- HG Verschuuren
  toxicologist; DOW Europe, Horgen (Switzerland)
- AAE Wibowo
  toxicologist; Coronel Institute, Amsterdam
- F de Wit
  occupational physician; Labour Inspectorate, Arnhem
- CA Bouwman, *scientific secretary*
  Health Council of the Netherlands, Den Haag
- ASAM van der Burght, *scientific secretary*
  Health Council of the Netherlands, Den Haag

The first draft of the present advisory report was prepared dr AAE Wibowo, from the Coronel Institute, Academic Medical Centre, University of Amsterdam, by contract with the Ministry of Social Affairs and Employment.

Secretarial assistance was provided by E Vandenbussche.
Lay-out: J van Kan.

Annex **C**

Comments on the public review draft

A draft of the present report was released in 1996 for public review. The following organisations and persons have commented on the draft document:

- A Kraak, Hoofddorp
- FM de Haas, LL.M., Elf Atochem, Rotterdam

Annex **D**

Abbreviations

bp	boiling point
EC_{50}	concentration at which a described effect is found in 50% of the exposed animals or at which the effect is decreased up to 50% of the control value
HBR-OEL	health based recommended occupational exposure limit
h	hour
IC_{50}	concentration at which inhibition of a certain function is found up to 50% of the control value
LC_{50}	lethal concentration for 50% of the exposed animals
LC_{lo}	lowest lethal concentration
LD_{50}	lethal dose for 50% of the exposed animals
LD_{lo}	lowest lethal dose
LOAEL	lowest observed adverse effect level
MAC	maximaal aanvaarde concentratie (maximal accepted concentration)
MAEL	minimal adverse effect level
MAK	Maximale Arbeitsplatz Konzentration
MOAEL	minimal observed adverse effect level
MTD	maximum tolerated dose
NAEL	no adverse effect level
NEL	no effect level
NOAEL	no observed adverse effect level
OEL	occupational exposure limit
PEL	permissible exposure limit
ppb	parts per billion (v/v)10^{-9}
ppm	parts per million (v/v)10^{-6}
RD_{50}	dose at which a 50% decrease of respiratory rate is observed
REL	recommended exposure limit

STEL	short term exposure limit
tgg	tijd gewogen gemiddelde
TLV	threshold limit value
TWA	time weighted average
V_{max}	maximal reaction velocity of an enzyme

Organisations

ACGIH	American Conference of Governmental and Industrial Hygienists
CEC	Commission of the European Communities
DECOS	Dutch Expert Committee on Occupational Standards
DFG	Deutsche Forschungsgemeinschaft
EPA	Environmental Protection Agency (USA)
FDA	Food and Drug Administration (USA)
HSE	Health and Safety Executive (UK)
IARC	International Agency for Research on Cancer (WHO)
INRS	Institut National de Recherche et de Sécurité (France)
NIOSH	National Institute for Occupational Safety and Health (USA)
NTP	National Toxicology Programme (USA)
OECD	Organisation for Economic Cooperation and Development
OSHA	Occupational Safety and Health Association (USA)
RTECS	Registry of Toxic Effects of Chemical Substances
SER	Social and Economic Council (Sociaal-Economische Raad NL)
WATCH	Working Group on the Assessment of Toxic Chemicals (UK)
WHO	World Health Organisation

Toxicological terms

bid	*bis in diem* (twice per day)
bw	body weight
CARA	chronic non-specific respiratory diseases
CHD	coronary heart disease
CNS	central nervous system
ECG	electrocardiogram
EEG	electro encephalogram
FCA	Freunds Complete Adjuvans
FEV	forced expiratory volume
FSH	follicle stimulating hormone
GD	gestation day(s)
GPMT	guinea pig maximisation test
GSH	glutathione
HLiA	hamster liver activated
IHD	ischaemic heart disease
im	intramuscular
ip	intraperitoneal
ipl	intrapleural
it	intratracheal
iv	intravenous
LH	lutheinising hormone
MAC	minimal alveolar concentration

MFO	mixed function oxidase
NA	not activated
PNS	peripheral nervous system
po	*per os* (= oral)
RBC	red blood cells
RLiA	rat liver activated
SCE	sister chromatid exchange
sc	subcutaneous
UDS	unscheduled DNA-synthesis

Statistical terms

GM	geometric mean
OR	Odds Ratio
RR	relative risk
SD	standard deviation
SEM	standard error of mean
SMR	standard mortality ratio

Analytical methods

AAS	atomic absorption spectroscopy
BEEL	biological equivalent exposure limit
BEI	biological exposure index
BEM	biological effect monitoring
BM	biological monitoring
ECD	electron capture detector
EM	environmental monitoring
FID	flame ionisation detector
GC	gas chromatography
GLC	gas liquid chromatography
GSC	gas solid chromatography
HPLC	high performance liquid chromatography
IR	infrared
MS	mass spectrometry
NMR	nuclear magnetic resonance
PAS	personal air sampling
TLC	thin layer chromatography
UV	ultraviolet

Additional abbreviations in the present report

GFR	Glomerular filtration rate

Annex **E**

DECOS-documents

DECOS has produced documents on the following substances.

To be ordered from the Health Council of the Netherlands:

Acetone cyanohydrin	1995/05WGD
p-Aramid fibres	1997/07WGD
Bisphenol A and its diglycidylether	1996/02WGD
Bromoethane	1998/10WGD
1,2-and t-Butanol	1994/10
Cadmium and inorganic cadmium compounds	1995/04WGD
Calculating cancer risk	1995/06WGD
Carbon disulphide	1994/08
Chlorine dioxide	1995/07WGD
p-Chloroaniline	1998/09WGD
4-Chloro-o-toluidine	1998/08WGD
Chromium and its inorganic compounds	1998/01WGD
Cresols	1998/15WGD
Copper sulphate	1999/01OSH
1,2-Dichloroethane	1997/01WGD
Diphenylamine	1997/05WGD
<Titeladv>	1998/03WGD
1,2-Epoxybutane	1998/11WGD
1,2-Ethanediamine	1996/03WGD
Ethyleneglycol ethers	1996/01WGD
Formamide and dimethylformamide	1995/08WGD
Hydrazinoethanol, phenylhydrazine, isoniazid, maleic hydrazide	1997/03WGD
Isopropyl acetate	1997/04WGD

Man made mineral fibers	1995/02WGD
Methyl Methacrylate	1994/09
Methacrylates. Ethyl methacrylate, n-butyl methacrylate and isobutyl methacrylate	1994/11
Methyl-t-butylether	1994/23
Methyl chloride	1995/01WGD
2-Nitrotoluene	1998/12WGD
Pentaerythritol	1997/06WGD
Phenol	1996/04WGD
o-Phenylenediamine	1998/06WGD
Piperidine	1997/08WGD
1- and 2-Propanol	1994/24
Propylene oxide	1997/02WGD
Ronidazole	1998/05WGD
Styrene	1998/07WGD
Quartz	1998/02WGD
1,1,1-Thrichloroethane	1995/03WGD
1,2,3-Trichloropropane	1994/25
1,2,3-Trichloropropane	1998/14WGD
Wood dust	1998/13WGD

The following documents, that were published before 1994, can be ordered from the Sdu Uitgeverij Den Haag.

Acetaldehyde	RA 6/92
Acrylaten	RA 13/87
Aflatoxine B1, B2, G1 en G2	RA 6/87
Allylglycidylether	RA 1/92
Amyl acetate	RA 4/90
Aniline	RA 2/89
Anorganisch Lood	RA 2/80
Anorganische Kwikzouten	RA 3/82
Arc welding fume particles not containing chromium and nikkel	RA 1/93
Arseenverbindingen (anorganische)	RA 2/84
Asbest	RA 1/84
Asbest, Evaluatie van risico op kanker bij beroepshalve blootstelling aan (aanvullend op RA 1/84)	RA 9/89
Benzeen	RA 5/89
Beryllium and beryllium compounds	RA 4/88
Blootstelling, Gezondheidskundige aspecten van het begrip en van het meten/schatten ervan	RA 8/90
Butadiene (1,3-)	RA 5/90
Cadmium	RA 5/80
Caprolactam	RA 4/84
Carbon monoxide	RA 7/92
Carbonylfluoride and PTFE pyrolysis products	RA 3/88
Carcinogene stoffen	RA 3/80

Chloor	RA 6/80
Chloroform	RA 7/87
ß-Chloroprene	RA 4/93
Chroom en chroomverbindingen	RA 6/85
Cyclohexane	RA 15/90
Cyclohexanol	RA 3/90
Cyclohexanone	RA 9/93
Dibroomethaan	RA 5/87
Dichloorethaan (1,1-)	RA 8/87
Diisocyanates	RA 3/91
Dimethyl- en diethylsulfaat	RA 12/90
Dimethylamine	RA 10/90
Dimethylbutane (2,2- & 2,3-)	RA 7/93
Dimethylhydrazine	RA 2/87
Dinitro-*ortho*-cresol (4,6-)	RA 4/87
Dioxaan (1,4-)	RA 1/87
Epichloorhydrine	RA 1/86
Ethylacrylate	RA 6/90
Ethylacetate	RA 10/91
Ethyl Methanesulphonate (EMS)	RA 4/89
Ethylamine	RA 7/90
Ethylbenzene	RA 9/91
Ethyleenoxide	RA 6/89
Fenylhydrazine	RA 2/87
Fluorcarbons (except FC11)	RA 15/87
Fluorine compounds (inorganic)	RA 1/89
Fluorine	RA 1/89
Formaldehyde	RA 3/87
Fosfine	RA 1/80
Fijn hinderlijk stof; gezondheidskundige aspecten van bijlage 3 bij de Nationale MAC-lijst 1989	RA 9/90
Gasoline	RA 3/92
Heptaan (n-)	RA 1/81
Heptane (n-)	RA 6/93
Hexaan (n-)	RA 11/87
Hexachlorobenzene	RA 2/88
Hexanone (2-)	RA 2/90
Hydrazine	RA 2/87
Hydrogenfluoride	RA 1/89
Hydroxyethylhydrazine	RA 12/87
Isopropylglycidylether	RA 1/92
Isopropoxyethanol (2-)	RA 2/87
Koolmonoxide (Carbon monoxide)	RA 2/79 (7/92)
Kwikalkylverbindingen - Korte keten	RA 5/82
Kwikverbindingen (Organische)	RA 4/82
Lachgas (Nitrous oxide)	RA 2/85 (2/92)
Lasrook (Arc welding fume.....nickel)	RA 1/93
Mangaan	RA 1/82

Metallisch Kwik	RA 5/81
1-Methoxypropanol-2	RA 5/93
2-Methoxypropanol-1	RA 5/93
1-Methoxypropylacetate-2	RA 5/93
2-Methoxypropylacetate-1	RA 5/93
Methylacrylate	RA 1/90
Methyleenchloride (Methylene chloride)	RA 1/83 (8/92)
Methyl ethyl ketone	RA 16/90
Methyl isobutyl ketone	RA 4/91
Methyl Methanesulphonate (MMS)	RA 4/89
Methylbromide	RA 13/90
Methylpentane (2- & 3-)	RA 7/93
Monochloorethaan	RA 2/82
Monoketones (7/8 carbon chain aliphatic)	RA 14/90
Nikkel en nikkelverbindingen	RA 3/85
Nitropropaan (2-)	RA 1/85
Nitrous oxide	RA 2/92
Ozone	RA 4/92
para-Dichloorbenzeen	RA 1/88
Pentaan	RA 2/81
Phthalate esters	RA 8/93
Phthalic anhydride	RA 3/89
Piperazine	RA 7/91
Polyvinyl chloride (PVC) dust	RA 2/93
Propoxyethanol (2-)	RA 12/87
Propoxyethylacetate (2-)	RA 12/87
Pyridine	RA 3/93
Selenium en -verbindingen	RA 7/89
Silicon dioxide, crystalline forms of	RA 5/92
Stikstofdioxide (Nitrogen dioxide)	RA 5/85
Styreen	RA 8/89
Talc dusts	RA 6/91
Tetrahydrofuran	RA 1/91
Thiourea	RA 11/90
Tolueen diisocyanaat	RA 4/80
Tolueen	RA 2/91
Trichloorethaan (1, 1, 1-)	RA 3/81
Trichloorethyleen	RA 3/83
Trichlorofluoromethane	RA 14/87
Triethylamine	RA 2/83
Trimethylamine	RA 9/87
Vadium metaal en anorganische verbindingen	RA 10/87
Wood dust	RA 8/91
Xylene	RA 5/91
Zwaveldioxide (sulphur dioxide)	RA 4/85