ETHYLENETHIOUREA

This substance was considered by previous working groups, in 1974 (IARC, 1974) and 1987 (IARC, 1987). Since that time, new data have become available, and these have been incorporated into the monograph and taken into consideration in the present evaluation.

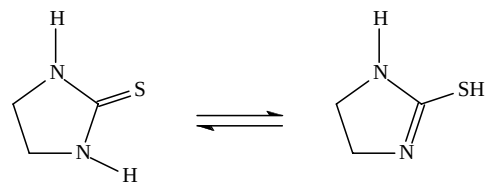
1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 96-45-7
Deleted CAS Reg. Nos: 96-46-8; 12261-94-8; 26856-29-1; 71836-04-9; 90613-75-5
Chem. Abstr. Name: 2-Imidazolidinethione
IUPAC Systematic Name: Imidazoline-2-thiol
Synonyms: 4,5-Dihydroimidazole-2(3*H*)-thione; 4,5-dihydro-2-mercaptoimidazole; *N*,*N*′-1,2-ethanediylthiourea; ethylenethiocarbamide; ethylene thiourea; 1,3-ethylenethiourea; 1,3-ethylene-2-thiourea; *N*,*N*′-ethylenethiourea; ETU; imidazolidinethione; 2-imidazoline-2-thiol; 2-mercapto-4,5-dihydroimidazole; mercaptoimidazoline; 2-mercaptoimidazoline; 2-mercapto-2-imidazoline; tetrahydro-2*H*-imidazole-2-thione; 2-thioimidazolidine

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_3H_6N_2S$ Relative molecular mass: 102.16

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Needles or prisms from alcohol (Lide & Milne, 1996; Budavari, 2000)
(*b*) *Melting-point*: 203 °C (Lide & Milne, 1996)
(*c*) *Spectroscopy data*: Infrared [prism (5619, 6556), grating (18092)], ultraviolet (4571), nuclear magnetic resonance [proton (7058), C-13 (5213)] and mass spectral data have been reported (Sadtler Research Laboratories, 1980; Lide & Milne, 1996).
(*d*) *Solubility*: Soluble in water (20 g/L at 30 °C), ethanol, methanol, ethylene glycol and pyridine; slightly soluble in dimethyl sulfoxide; insoluble in acetone, benzene, chloroform, diethyl ether and ligroin (Lide & Milne, 1996; Budavari, 2000)

1.1.4 *Technical products and impurities*

Trade names for ethylenethiourea include Accel 22, Accel 22S, Akrochem ETU-22, END 75, ETC, Mercazin I, NA-22, Nocceler 22, Pennac CRA, Rhenogran ETU, Robac 22, Rodanin S 62, Sanceller 22, Sanceller 22C, Sanceller 22S, Soxinol 22, Thiate N, Vulkacit NPV/C and Warecure C.

1.1.5 *Analysis*

Methods for the analysis of ethylenethiourea in fresh, baked or frozen food commodities (fruit, vegetables, canned goods, soups), beverages (milk, beer, juice), water (finished drinking-, surface, ground-, river), cigarette smoke condensate, blood serum, urine and formulated fungicides have been reported. The methods include thin-layer chromatography with liquid scintillation counting, micellar electrokinetic capillary chromatography, spectrophotometry, gas chromatography with flame photometric, electron capture, nitrogen–phosphorus or negative-ion chemical-ionization mass spectrometry detection, liquid chromatography with mass spectrometry or ultraviolet detection, high-performance liquid chromatography (HPLC) with amperometric, chemiluminescent nitrogen, diode-array or electrochemical detection, and reversed-phase HPLC with ultraviolet detection (Autio, 1983; Prince, 1985; Sonobe & Tanaka, 1986; Krause & Wang, 1988; Kurttio *et al*., 1988; Longbottom *et al*., 1993; van der Poll *et al*., 1993; Walash *et al*., 1993; Beneventi *et al*., 1994; Maruyama, 1994; Meiring & de Jong, 1994; Ahmad *et al*., 1995; Dubey *et al*., 1997; Lo & Hsiao, 1997; Neicheva *et al*., 1997; do Nascimento *et al*., 1997; Fujinari, 1998; AOAC International, 1999a,b; Knio *et al*., 2000; Picó *et al*., 2000). Earlier methods for the determination of ethylenethiourea residues have been reviewed (Bottomley *et al*., 1985).

1.2 Production

Ethylenethiourea can be made from ethylenediamine and carbon disulfide (Ohm, 1997).

Information available in 2000 indicated that ethylenethiourea was manufactured by three companies each in China, France and Japan, two companies in Germany and one company each in Brazil, India, Italy, the Netherlands and Switzerland (CIS Information Services, 2000).

1.3 Use

Ethylenethiourea is used mainly in the rubber industry as an accelerator for the vulcanization of polychloroprene (neoprene) and other rubbers. The use of thioureas is decreasing, and it is supplied preferably as polymer-bound granulates, which effectively prevent exposure to and inhalation of thiourea dust (Engels, 1993; Ohm, 1997; Budavari, 2000).

1.4 Occurrence

Ethylenethiourea is an environmental degradation product, a metabolite and an impurity in ethylenebisdithiocarbamate fungicides such as mancozeb, maneb and zineb. Mancozeb and maneb have a wide range of approved uses on agricultural and horticultural crops in many countries. Ethylenebisdithiocarbamate residues in foods are readily converted to ethylenethiourea during storage (Kurttio *et al.*, 1990) and when processing includes a heating step (cooking or industrial processing) (FAO/WHO, 1993a; IPCS/INCHEM, 1993; Aprea *et al.*, 1996; Budavari, 2000).

1.4.1 *Occupational exposure*

According to the 1981–83 National Occupational Exposure Survey (National Institute for Occupational Safety and Health, 2000), about 10 700 workers in the USA were potentially exposed to ethylenethiourea. Exposure occurred mainly during the manufacture of fabricated metal products (5000 exposed), the manufacture of machinery (3300) and in rubber mills (2300). The occupational groups exposed included grinding, abrading, buffing and polishing machine operators (2700 exposed), metal-plating-machine operators (1900) and moulding- and casting-machine operators (1300). Farmers, who may have been exposed to ethylenethiourea through the use of ethylenebisdithiocarbamate fungicides, were not included in the survey. According to the Finnish Register of Employees Exposed to Carcinogens, 47 chemical process workers and cleaners were exposed to ethylenethiourea in Finland in 1997. Most Finnish farmers are self-employed and therefore not covered by this register (Savela *et al*., 1999).

The average concentration of ethylenethiourea in the breathing zone was 0.14 μg/m^3 for Finnish potato-field workers and 0.60 μg/m^3 for pine nursery workers during spraying of an ethylenebisdithiocarbamate, maneb. Higher short-term concentrations (0.87 μg/m^3 and 1.81 μg/m^3 respectively) were measured during weighing of the pesticide. The average concentration of ethylenethiourea in the urine was 1.5 μg/L for potato-field workers and 0.9 μg/L for pine nursery workers 3 h after exposure (Savolainen *et al.*, 1989). In another study in Finland on potato-farmers spraying maneb or mancozeb, 0.004–3.3 μg/m^3 were found in the breathing zone of farmers and 0.006–0.8 μg/m^3 in tractor cabins. The urine samples contained < 0.2–11.8 μg/L ethylenethiourea (Kurttio *et al.*, 1990).

The concentration of ethylenethiourea in a rubber mill in the USA ranged from not detected to 1100 μg/m^3 when ethylenethiourea was used in a dry powder form as an accelerator for curing neoprene rubber. When the powder was replaced by a 75% dispersion in a rubber binder, the concentration of ethylenethiourea in the air dropped to not detected to 29 μg/m^3 (Salisbury & Lybarger, 1977).

1.4.2 *Environmental occurrence*

Within a Food and Drug Administration monitoring programme in the USA, 864 samples of baby foods were monitored for pesticide residues. Ethylenethiourea residues were detected in 65 samples at concentrations ranging from traces to 0.06 mg/kg (Yess *et al.*, 1993).

In 1989–90 in the USA, a large survey of food items (approximately 300 samples each of 19 raw and processed commodities) was conducted for dithiocarbamate and ethylenethiourea residues. No measurable residues of ethylenethiourea (limit of detection, 0.001 mg/kg) were found in 82% of the samples. All of the concentrations detected were < 0.1 mg/kg (FAO/WHO, 1993a).

Ethylenethiourea was not detected (< 0.005 mg/kg) in any of 100 commercial grape juice samples in the USA taken from producers using grapes from areas where dithiocarbamate fungicides were used (FAO/WHO, 1993a).

As part of a study of new analytical techniques, Walash *et al*. (1993) reported that the disappearance of maneb and zineb sprayed on cucumbers and tomatoes grown in a greenhouse followed first-order kinetics.

In 1988 and 1989, the concentrations of residues of ethylenebisdithiocarbamate, chlorothalonil and anilazine on raw, unwashed, unpeeled processing tomatoes in field experiments represented 16–25% of those tolerated by the Environmental Protection Agency in the USA. The concentrations of ethylenethiourea were at or below detection limit (< 0.01 mg/L) in tomato juice processed from field-grown tomatoes in both years (Precheur *et al.*, 1992).

The efficiency of vegetable washing was evaluated by measuring the concentrations of residues of ethylenethiourea in canned products. Collard and spinach retained more carbamate residues in the field than other green vegetables. Spinach

retained more residues than the other leafy green vegetables, regardless of the washing treatment. With the exception of mustard, neither mild nor strong detergent removed significantly more epicuticular waxes in leafy green vegetables than did water (Gonzalez *et al.*, 1990).

Urinary ethylenethiourea concentrations were measured in the populations of several urban and rural regions in Italy in 1994 and 1995. Measurable concentrations were found in an average of 24% of the urban population (range, 0.8–8.3; mean, 2.7 μg/g of creatinine) and 37% of the rural population (range, 0.9–61.4; mean, 9.1 μg/g creatinine). The concentrations were increased by smoking and wine-drinking. The estimated intake of ethylenethiourea from several food commodities (mean values in μg/day per capita ± SD) were: wine, 6.03 ± 4.62; vegetables, 18.53 ± 40.16; whole fruit, 155.33 ± 122.41; and fruit pulp, 31.07 ± 24.48 (Aprea *et al.*, 1996).

As zineb, maneb and mancozeb are used as fungicides in vineyards (IARC, 1976), trace concentrations of ethylenethiourea can occur in wine (Cabras *et al.*, 1987). For example, 5–10 μg/L ethylenethiourea were found in all of 10 local wine samples in Italy (Aprea *et al.*, 1996).

Ethylenethiourea may occur also in cigarette smoke. The condensate of four of 12 brands of cigarettes contained 8–27 ng/cigarette of ethylenethiourea, owing to the use of ethylenebisdithiocarbamate on tobacco crops (Autio, 1983).

1.5 Regulations and guidelines

In Germany, ethylenethiourea is classified as 3B ('substances for which in-vitro or animal studies have yielded evidence of carcinogenic effects that is not sufficient for classification of the substance in one of the other categories. Further studies are required before a final decision can be made. A MAK value can be established provided no genotoxic effects have been detected') (Deutsche Forschungsgemeinschaft, 2000). Finland, France, Sweden and the USA (National Institute for Occupational Safety and Health) list ethylenethiourea as a carcinogen; Finland has set a time-weighted average occupational exposure limit of 0.2 mg/m^3 and a short-term exposure limit of 0.6 mg/m^3 for ethylenethiourea (American Conference of Governmental Industrial Hygienists, 2000).

Ethylenethiourea was reviewed in conjunction with the ethylenebisdithiocarbamates by the Joint FAO/WHO Meeting on Pesticide Residues several times between 1963 and 1993. In 1993, the Joint Meeting established an acceptable daily intake for ethylenethiourea of 0–0.004 mg/kg bw (FAO/WHO, 1993b; WHO, 1999).

2. Studies of Cancer in Humans

2.1 Cohort study

A list of 1929 workers at several large rubber manufacturing firms where ethylenethiourea was used and at a firm producing ethylenethiourea in England was drawn up from employment records (Smith, 1976). According to the records of the Birmingham Cancer Registry for the period 1957–71, none of the workers developed thyroid cancer. [The Working Group noted that the lack of details on methods, including the number of expected cases, makes it difficult to assess the relevance of this finding.]

Although workers in the rubber industry and pesticide applicators may be exposed to ethylenethiourea, no specific mention of this compound was found in epidemiological studies of the cancer risks of these populations.

3. Studies of Cancer in Experimental Animals

3.1 Oral administration

Mouse: In a preliminary report of a screening study, groups of 18 male and 18 female hybrid mice of the (C57BL/6 × C3H/Anf)F_1 (B6C3F_1) and (C57BL/6 × AKR)F_1 (B6AKF_1) strains, 7 days of age, were given doses of 0 (control) or 215 mg/kg bw commercial-grade ethylenethiourea [purity not specified] daily in 0.5% gelatin in water by gavage for 3 weeks. The dose determined at 7 days of age was not adjusted for body weight. The mice were weaned at 4 weeks of age, and the chemical without vehicle was mixed into the diet at a concentration of 0 (control) or 646 mg/kg and provided *ad libitum* from 4 weeks to approximately 18 months. The concentration of the compound in the diet was calculated from the weight and food consumption of the 4-week-old mice to be approximately the maximum tolerated dose on a mg/kg bw basis. The same concentration was maintained throughout the duration of the study up to 82–83 weeks of age. The incidence of 'hepatomas' was 14/16 (male) and 18/18 (female) in treated B6C3F_1 mice and 18/18 (male) and 9/16 (female) in treated B6AKF_1 mice, with incidences in the pooled control groups of 8/79 (male) and 0/87 (female) for the B6C3F_1 mice and 5/90 (male) and 1/82 (female) for the B6AKF_1 mice. The increases in incidences of hepatomas in male and female mice of both strains were statistically significant ($p < 0.01$) (Innes *et al*., 1969).

The carcinogenic potential of ethylenethiourea was evaluated during and after perinatal exposure (*in utero* and throughout suckling). Female C57BL/6 mice, 10–11 weeks of age (F_0 generation), were fed a diet containing 0, 33, 110 or 330 mg/kg ethylenethiourea for 1 week before breeding. After mating with previously unexposed male C3H/HeN mice, all the females were continued on the diets containing ethylenethiourea. On day 7 *post partum*, the litters (F_1 generation) were standardized to a maximum of

eight, weaned on day 28 and separated by sex. Up to 8 weeks of age, the litters were exposed to ethylenethiourea at the same concentrations in the diet as those given to their dams; at approximately 8 weeks of age, the pups were divided into groups of 50 animals per sex and exposed to the adult concentrations of 0, 330 and 1000 mg/kg of diet for 2 years. The F_0:F_1 treatments were thus 0:0, 0:330, 0:1000, 330:0, 330:330, 330:1000, 33:100 and 110:330 mg/kg of diet. The tumour incidences in the various groups are shown in Table 1. Significant ($p < 0.01$) increases were found in the incidences of liver tumours in males and females at 330 mg/kg of diet, with or without perinatal exposure. Significant ($p < 0.01$) increases in the incidences of thyroid follicular-cell tumours were observed in females at 330 mg/kg of diet with perinatal exposure and in males and females at 1000 mg/kg of diet with or without perinatal exposure. Significant ($p < 0.01$) increases in the incidences of anterior pituitary tumours were observed in females at 330:330 and 330:1000 mg/kg of diet and in F_1 males and F_1 females at 0:1000 mg/kg of diet. The incidences of tumours were generally similar with and without perinatal exposure, except that the incidences of thyroid and anterior pituitary tumours in the females were higher after perinatal exposure (Chhabra *et al*., 1992; National Toxicology Program, 1992).

Table 1. Incidences of neoplasms in B6C3F_1 mice exposed to ethylenethiourea in the diet with or without perinatal exposure

Concentration of ethylenethiourea (F_0:F_1) (mg/kg of diet)	Hepatocellular adenoma and carcinoma combined		Thyroid follicular-cell adenoma and carcinoma combined		Anterior pituitary adenoma and carcinoma combined	
	Males	Females	Males	Females	Males	Females
Adult exposure only						
0:0	20/49	4/50	1/50	0/50	0/44	11/47
0:330	32/50*	44/50**	1/49	2/50	0/42	19/49
0:1000	46/50**	48/50**	29/50**	38/50**	8/41**	26/49**
Perinatal and adult exposure						
33:100	9/33	4/28	1/47	1/29	0/28	2/28
110:330	26/47	46/50**	1/47	5/50*	0/41	14/48
330:330	34/49*	46/50**	2/48	10/49**	0/45	26/47**
330:1000	47/49**	49/50**	35/49**	38/50**	4/39	24/47**
Perinatal exposure only						
330:0	13/49	5/49	1/46	1/49	0/42	11/48

From Chhabra *et al*. (1992); National Toxicology Program (1992). Incidences are numbers of lesions observed/number of animals

* $p < 0.05$ versus 0:0 group (logistic regression test)

** $p < 0.01$ versus 0:0 group (logistic regression test)

Rat: Groups of 26 male and 26 female Sprague-Dawley (Crl:CD®) rats, 5–6 weeks of age, were fed diets containing 175 or 350 mg/kg technical-grade ethylenethiourea (97% pure) for 18 months. Five rats per dose group were killed and necropsied at 18 months, and the remaining rats at the two concentrations were continued on control diet for up to a further 6 months, for a total of up to 24 months. Thyroid (follicular or papillary) carcinomas were observed in 0/30 and 0/30 male and female controls, 2/26 and 2/26 males and females at the lower concentration and 15/26 and 6/26 males and females at the higher concentration, respectively (Ulland *et al*., 1972; Weisburger *et al*., 1981).

Groups of 11–13 male and 9–12 female Sprague-Dawley (Crl:CD®) rats, approximately 5 weeks of age, were given diets containing 0 (control), 5, 25, 125, 250 or 500 mg/kg ethylenethiourea [purity not specified] *ad libitum* for up to 12 months and were evaluated for histological changes. Thyroid follicular-cell adenocarcinomas were observed in 3/13 males at 250 mg/kg of diet and 10/13 males and 5/12 females at 500 mg/kg of diet at 12 months. No neoplastic changes were found in the other groups, including the controls (Graham *et al*., 1973). In a second study, which was a continuation of the previous one, the carcinogenic potential of the same concentrations when given for 12–24 months was evaluated. When the results for male and female rats were combined, the incidences of thyroid tumours (adenomas and adenocarcinomas/carcinomas) were 4/72 controls, 37/69 rats at 250 mg/kg of diet and 65/70 rats at 500 mg/kg of diet. A few thyroid tumours occurred in other groups of 72–75 rats, including that given no ethylenethiourea (Graham *et al*., 1975).

Five groups of 20 male and 20 female rats [strain or stock and age not specified] were fed diets containing ethylenethiourea at 0 (control), 5,17, 60 or 200 mg/kg for 24 months. There was a strong negative association between food consumption and bodyweight gain and dietary concentration, the decreases in food consumption and bodyweight gain being > 10% at the two higher concentrations. The incidences of thyroid tumours were 0, 0, 5.9, 42.1 ($p < 0.01$) and 82.4% ($p < 0.001$) in males and 5.3, 6.3, 18.8, 22.2 and 56.3% ($p < 0.001$) in females at 0, 5, 17, 60 and 200 mg/kg of diet ethylenethiourea, respectively (Gak *et al*., 1976).

Female Fischer 344 rats, 10–11 weeks of age (F_0 generation), were fed a diet containing 0, 9, 30 or 90 mg/kg ethylenethiourea for 1 week before breeding. After mating with previously unexposed male Fischer 344 rats, all the females were continued on their previous diets. On day 4 *post partum*, the litters (F_1 generation) were standardized to a maximum of eight and weaned on day 28. The pups continued to be exposed at the concentrations given to their dams until they were 8 weeks of age. The pups were separated by sex at weaning, and at approximately 8 weeks of age were divided into groups of 50 animals per sex and exposed to the adult dietary concentrations of 0, 25, 83 and 250 mg/kg for 2 years. The F_0:F_1 treatments were thus 0:0 (control), 0:83, 0:250, 90:0, 90:83, 9:250, 30:83, and 9:25 mg/kg of diet. The incidences of thyroid tumours in the various groups are shown in Table 2. Significant increases in the incidences of thyroid follicular-cell tumours were observed in males at 83 and 250 mg/kg of diet, with or

Table 2. Incidences of thyroid follicular-cell adenoma and carcinoma combined in Fischer 344 rats exposed to ethylenethiourea in the diet with or without perinatal exposure

Concentration of ethylenethiourea (F_0:F_1) (mg/kg of diet)	No. of lesions observed/number of animals	
	Males	Females
Adult exposure only		
0:0	1/49	3/50
0:83	12/46*	7/44
0:250	37/50*	30/49*
Perinatal and adult exposures		
9:25	3/46	1/49
30:83	14/47*	6/47
90:83	13/50*	9/47
90:250	48/50*	37/50*
Perinatal exposure only		
90:0	4/49	3/50

From Chhabra *et al.* (1992); National Toxicology Program (1992)
* $p < 0.01$ versus 0:0 group (logistic regression test)

without perinatal exposure, and in females at 250 mg/kg of diet, with or without perinatal exposure when compared with their respective control groups. Marginally significant ($p < 0.05$) increases in the incidence of Zymbal gland tumours were observed in males (5/50) at 90:250 mg/kg of diet and in only 1/50 control males. The incidences of tumours were generally similar with and without perinatal exposure, except that the incidences of thyroid tumours were higher with perinatal exposure at the higher concentrations (Chhabra *et al*., 1992; National Toxicology Program, 1992).

Hamster: Five groups of 20 male and 20 female hamsters [strain or stock and age not specified] were fed diets containing ethylenethiourea at 0 (control), 5, 17, 60 or 200 mg/kg for 20 months. There was a strong negative association between food consumption and body-weight gain and dietary concentration, the decreases in food consumption and body-weight gain being > 10% at the two higher concentrations. No carcinogenic effects were observed (Gak *et al*., 1976).

3.2 Administration with known carcinogens and modifying factors

Mouse: Groups of 30 male and 30 female ICR mice, 5 weeks of age, were given a prescribed amount of ethylenethiourea (obtained from commercial sources and purified by recrystallization twice from 1:1 ethanol:water or methanol) and/or sodium nitrite by gavage at a dosage volume of 0.1 mL/10 g bw distilled water. The doses of ethylenethiourea/sodium nitrite were 0/0 (control), 100/0, 0/70, 25/17.5, 50/35 or 100/70 mg/kg bw per week. The animals were treated once a week for 10 weeks, and the study was terminated 18 months after the first administration. Significant increases ($p < 0.05$) in the incidences of tumours at various sites, including the lung, forestomach and uterus (see Table 3), were observed with the combinations of 50/35 and 100/70 mg/kg bw per week (Yoshida *et al*., 1993).

Table 3. Tumour incidences in ICR mice treated with ethylenethiourea and sodium nitrite

Neoplasm	Tumour incidence per dose group (mg/kg bw per week)					
	Males			Females[a]		
	0/0	50/35	100/70	0/0	50/35	100/70
Lymphoma	3/30	8/30	13/30*	6/30	12/30	19/30*
Lung (adenoma/adenocarcinoma)[a]	9/30	22/30*	25/30*	3/30	16/30*	21/30*
Forestomach (squamous papilloma/ carcinoma)	0/30	4/30	12/30*	0/30	2/30	8/30*
Harderian gland (adenoma)	3/30	2/30	9/30	0/30	3/30	7/30*
Uterus (adenocarcinoma)				0/30	3/30	6/30*

From Yoshida *et al*. (1993)

* Significantly different from controls ($p < 0.05$)

[a] A significantly increased incidence (12/30) of lung tumours was also observed with the 25/17.5 combination in females

The uterine adenocarcinomas induced in the above study prompted two further studies.

Groups of female ICR mice, 5 weeks of age, were given distilled water (40 mice) or a combination of 100 mg/kg bw ethylenethiourea (purity, > 95%) and 70 mg/kg bw sodium nitrite (purity, > 98%) by gavage (90 mice) in distilled water once a week for up to 6 months. The experiment was terminated at 12 months. Small groups of control and treated mice were killed sequentially between 1 and 12 months of the study. Significantly higher incidences ($p < 0.05$) of endometrial adenocarcinomas (17/40) and stromal polyps (23/40) were observed in treated mice after 10–12 months than in control mice (0/13 adenocarcinomas and 2/13 stromal polyps) (Yoshida *et al*., 1994).

Groups of 20 female ICR mice, aged 1, 6 and 12 months, were given the same treatment as described above for 6 months, followed by a withdrawal period of 3 months, at which time all surviving mice were necropsied. Age-matched control groups of 10 mice per group were gavaged with distilled water for 6 months. All mice were evaluated for uterine lesions. The incidences of endometrial adenocarcinoma were 1/20, 8/20 and 4/20 in treated 1-, 6- and 12-month-old mice, respectively, with none in age-matched control groups, but the incidence was significantly ($p < 0.05$) higher only in the 6-month-old group. The incidences of endometrial stromal polyps were 5/20, 13/20 ($p < 0.05$) and 10/20 in the treated 1-, 6- and 12-month-old mice, with 0/10, 1/10 and 2/10 in the respective age-matched control groups. The authors concluded that adult mice are more susceptible than young or old mice to induction of endometrial adenocarcinoma by the reaction product of ethylenethiourea and sodium nitrite, *N*-nitrosoethylenethiourea (Yoshida *et al*., 1996).

Rat: The initiating and promoting effects of oral administration of ethylenethiourea and sodium nitrite were investigated in female Donryu rats, which are predisposed to a high incidence of endometrial adenocarcinomas (approximately 35% by 120 weeks of age; Nagaoka *et al*., 1990) probably due to a high oestrogen:progesterone ratio (imbalance). Groups of 21–37 female Donryu rats, 10 weeks of age, were treated as follows: group 1 received a single intrauterine injection of polyethylene glycol, followed by oral gavage with distilled water at weekly intervals from weeks 11 to 51 of age (vehicle control); group 2 received a single intrauterine injection of 15 mg/kg bw *N*-ethyl-*N*-nitrosourea (ENU) in polyethylene glycol; group 3 received a single intrauterine injection of polyethylene glycol followed by oral gavage with a combination of 80 mg/kg bw ethylenethiourea and 50 mg/kg bw sodium nitrite in water at weekly intervals from week 11 to 51 of age; and group 4 received a single intrauterine injection of 15 mg/kg bw ENU followed by oral gavage with a combination of 80 mg/kg bw ethylenethiourea and 50 mg/kg bw sodium nitrite in water at weekly intervals from week 11 to 51 of age. The study was terminated at 52 weeks of age, and the tissues were evaluated for histological changes. The incidences of endometrial adenocarcinomas were 0/21, 6/21 ($p < 0.01$; significantly different from group 1), 4/31 and 21/37 ($p < 0.001$; significantly different from groups 1 and 3) in groups 1–4, respectively, suggesting that concurrent administration of ethylenethiourea and sodium nitrite promoted ENU-initiated endometrial adenocarcinoma (Nishiyama *et al*., 1998).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

Urinary excretion of ethylenethiourea was monitored in non-smoking male volunteers given a diet with no detectable ethylenethiourea except in wine (8.8 μg/L) for 8 days. An average of 48.3% of the ethylenethiourea ingested from wine was excreted unmodified in the urine (Aprea *et al*., 1997).

4.1.2 *Experimental systems*

Imidazoline, ethylene urea, 4-imidazolin-2-one(imidazolone) and unchanged ethylenethiourea were identified in the 24-h urine of male Sprague-Dawley rats after oral administration of 4 mg/kg bw [^{14}C]ethylenethiourea. In two female cats given the same dose, a half-time of 3.5 h was determined, and ethylenethiourea, ethylene urea and *S*-methyl ethylenethiourea were present in the 24-h urine. *S*-Methyl ethylenethiourea comprised 64% of the total radiolabel in urine. In-vitro metabolism by cat and rat liver microsomes also produced ethylene urea, imidazoline and other unidentified compounds. When cat liver supernatant contained *S*-adenosyl methionine, *S*-methyl ethylenethiourea was produced (Iverson *et al*., 1980). Using microsomes from male Sprague-Dawley rats, Decker and Doerge (1991) showed that, under normal physiological conditions, the reactive species from flavin-monooxygenase and cytochrome P450 metabolism of ethylenethiourea are sequestered by endogenous glutathione. The main metabolite of [^{14}C]ethylenethiourea, formed *in vivo* after treatment of male NMRI mice with an oral dose of 67 mg/kg bw or *in vitro* with mouse liver microsomes, was 2-imidazolin-2-yl sulfenate (Savolainen & Pyysalo, 1979). A metabolite of ethylenethiourea detected in female rat plasma was identified as 1-methylthiourea (Kobayashi *et al*., 1982).

After ethylenethiourea was administered once orally at 200 mg/kg bw to Wistar rats on day 12 of gestation, the concentration in maternal plasma and amniotic fluid peaked at about 2 h and disappeared within 48 h. In the embryos, the concentration peaked after only 30 min and disappeared within 48 h (Iwase *et al*., 1996). In pregnant Wistar rats given a single oral dose of 240 mg/kg bw [^{14}C]ethylenethiourea, maternal blood maintained peak radiolabel concentrations for 2 h; the distribution was equal among maternal tissues but lower in embryos. Twenty-four hours after treatment, the radiolabel had been cleared and 72.8% had been excreted in the urine. The elution patterns suggested very little metabolism of the parent compound (Ruddick *et al*., 1975). When Wistar rats were given 100 mg/kg bw [^{14}C]ethylenethiourea orally on day 12 of gestation, the compound was readily absorbed, the concentration reaching a

maximum in maternal blood within 2 h. Ethylenethiourea was distributed throughout the maternal system and the embryo. Accumulation was noted in the thyroid. The major elimination route was the urine (Kato *et al*., 1976). Swiss mice and Wistar rats were treated by gavage on gestational day 15 with 240 mg/kg bw [^{14}C]ethylenethiourea (mice) or [^{35}S]ethylenethiourea (rats). The maternal and fetal concentrations of ethylenethiourea in tissues were similar in the two species 3 h after treatment, but the mice eliminated ethylenethiourea more rapidly, with a half-time of 5.5 h in mice and 9.4 h in rats (Ruddick *et al*., 1977).

Two female rhesus monkeys (*Macaca mulatta*) and four Sprague-Dawley rats were given [^{14}C]ethylenethiourea at 40 mg/kg bw by gastric intubation, and excretion was monitored for 48 h. The major excretion route was urine. The amount retained in tissues at 48 h was much higher in the two monkeys (21 and 28%) than in the rats (1%) (Allen *et al*., 1978).

4.2 Toxic effects

4.2.1 *Humans*

A 53-year-old woman reported allergic contact dermatitis after exposure to ethylenethiourea used as a rubber additive (Bruze & Fregert, 1983).

A group of 49 male workers without protective equipment used backpack sprayers to apply ethylenebisdithiocarbamate fungicides in which ethylenethiourea was found as a contaminant and metabolic product. They were found to have a marginal increase in the serum concentration of thyroid-stimulating hormone but no change in that of thyroxine (T4) (Steenland *et al*., 1997).

Over a period of 3 years, five workers involved in mixing ethylenethiourea into monomer rubber showed decreased serum concentrations (by approximately 20%) of T4. One had an increased concentration of thyroid-stimulating hormone on two occasions [about 10-fold], but he was found to have premyxoedema (Smith, 1984).

4.2.2 *Experimental systems*

Osborne-Mendel rats were fed diets containing ethylenethiourea at a concentration of of 50, 100, 500 or 750 mg/kg for 30, 60, 90 or 120 days. Rats at the two higher concentrations showed decreased body weight and hyperplasia of the thyroid at all times. The thyroid:body weight ratios were increased at concentrations of 100, 500 and 750 mg/kg of diet at 30 and 60 days, at the two higher concentrations at 90 days and at all concentrations at 120 days. Decreased iodine uptake was measured 24 h after injection at concentrations of 100, 500 and 750 mg/kg of diet at all times (Graham & Hansen, 1972).

Male Sprague-Dawley rats given diets containing 125 or 625 mg/kg ethylenethiourea for up to 90 days had decreased serum concentrations of triiodothyronine and T4 [by 65% and 60%, respectively] and, at 625 mg/kg of diet, decreased iodide uptake

[by 35%] in the thyroid (Freudenthal *et al*., 1977). Male Sprague-Dawley rats given ethylenethiourea in drinking-water at 500 mg/L for 4 months had altered hepatic morphology, increased smooth endoplasmic reticulum, decreased rough endoplasmic reticulum and relocation of microbodies and mitochondria to the periphery of the smooth endoplasmic reticulum (Moller *et al*., 1986). When male and female Sprague-Dawley rats were given ethylenethiourea in the diet for 7 weeks and then removed to control diet, the increases in relative and absolute thyroid weights (at 75 and 100 mg/kg of diet) and the decrease in T4 blood concentration (at 150 mg/kg bw) were partially reversed (Arnold *et al*., 1983).

Male Wistar rats given ethylenethiourea in the drinking-water at 100–300 mg/L [corresponding to 10.6–23.4 mg/kg bw per day] for 28 days showed reduced secretion of T4 and triiodothyronine and a 10-fold increase in the secretion of thyroid-stimulating hormone over that in controls. Ultrastructural changes were also found in the thyroid, with an increased number of myelin bodies, dilatation of the rough endoplasmic reticulum and increased vacuolization in the epithelial cells of thyroid follicles (Kurttio *et al*., 1986). Alterations in renal proximal tubule epithelial cells were seen in male Wistar rats given a high concentration of ethylenethiourea in the drinking-water (300 mg/L) for 28 days. Continuous oral administration had only minor effects on renal function (Kurttio *et al*., 1991).

Both male and female Charles River rats showed decreased body weight and body-weight gain when fed diets containing 250 or 500 mg/kg ethylenethiourea for 2–12 months. The body weight of the female rats was also decreased at 125 mg/kg of diet. Iodine uptake was decreased in male rats after 12 months at 500 mg/kg of diet. Females had an initial decrease in iodine uptake at a dose of 125 or 500 mg/kg of diet at 6 months, but by 12 months the uptake had increased even on diets containing 125, 250 or 500 mg/kg (Graham *et al*., 1973).

Sprague-Dawley rats were given ethylenethiourea as a single intraperitoneal injection of 2.5 or 250 mg/kg bw, by gavage for 3 days at 5 or 250 mg/kg bw per day or in the diet for 3 weeks at 5 or 250 mg/kg of diet. The livers of the animals were morphologically normal, and no changes in hepatic RNA synthesis occurred (Austin & Moyer, 1979).

Chinese hamster ovary cells transfected with the human thyroid peroxidase (*TPO*) gene were exposed to ethylenethiourea. The oxidative activity of the enzyme was inhibited at 50 μmol/L ethylenethiourea, and its iodinating activity was blocked at 5 μmol/L (Marinovich *et al*., 1997).

In vitro, ethylenethiourea inhibited thyroid peroxidase, the enzyme that catalyses the iodination and coupling of the tyrosine residues needed for the synthesis of triiodothyronine and T4, by interacting with the iodinated enzyme intermediate. Once the ethylenethiourea was depleted, normal enzymatic activity returned (Doerge & Takazawa, 1989).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

A retrospective study of women who had been employed in the manufacture of rubber containing ethylenethiourea was reported (Smith, 1976). The potential participants were all 699 women of child-bearing age who had left employment at the factory between 1963 and 1971. Of these, 255 who had given birth to 420 children were traced. Of these women, 59 had been employed in the rubber plant at the time of their first pregnancy, and none had given birth to an abnormal child. Of the 420 children, 11 had malformations. Three of these had been born before the employment of their mother and eight had been born more than 1 year after their mothers' employment.

4.3.2 *Experimental systems*

The teratogenicity of ethylenethiourea has been reviewed (Khera, 1987).

(*a*) *General developmental toxicity*

As reported in an abstract, rats [strain and group size not specified] were given 0, 10, 20, 40 or 80 mg/kg bw per day ethylenethiourea by gavage either from 21 days before gestation to day 15 or on days 6–15 or 7–20 of gestation. A variety of malformations was observed, with minimal effects noted at the lowest dose. Rabbits similarly exposed on days 7–20 of gestation were reported to have an increased incidence of resorptions and decreased brain weight at 80 mg/kg bw per day (Khera, 1973).

The teratogenic effects of ethylenethiourea were evaluated in groups of 12–29 Sprague-Dawley rats, 31–33 CD-1 mice, 15–19 golden hamsters and three to five Hartley guinea-pigs exposed daily by oral gavage on days 7–21, 7–16, 5–10 and 7–25 of gestation, respectively, to a dose of 0, 5, 10, 20, 30, 40 or 80 mg/kg bw per day, 0, 100 or 200 mg/kg bw per day, 0, 75, 150 or 300 mg/kg bw per day and 0, 50 or 100 mg/kg bw per day, respectively. The fetuses were examined at the end of gestation for external, internal and skeletal malformations. Ethylenethiourea was toxic to the pregnant rats at 80 mg/kg bw per day, while a variety of malformations (e.g., hydrocephalus, encephalocoele, cleft palate, kyphosis and limb and digital defects) were observed at doses ≥ 20 mg/kg bw per day; fetal body weights were reduced at doses as low as 10 mg/kg bw per day. In mice, the maternal liver weights were increased at the two highest doses; the only significant fetal effect was an increased incidence of supernumerary ribs at 200 mg/kg bw per day. No significant maternal or fetal effects were seen in hamsters or guinea-pigs. Other groups of 11–13 rats received 0, 20, 25 or 30 mg/kg bw per day ethylenethiourea on gestation day 7; they delivered their offspring, and exposure was continued until lactation day 15. The offspring were tested for a variety of indicators of reflex development, and the motor activity of males was recorded in an open-field device for 4 min over 2 consecutive days at 6 weeks of age. There were no effects on litter size at birth, but 6/13 litters of dams at the highest dose failed to nurse, and 40%

of the surviving offspring had developed hydrocephaly by day 45. There were no treatment-related effects on the offspring body weights, startle or righting reflex development or eye opening, but there was a dose-related increase in defaecation in the open-field test on days 1 and 2 and in activity on day 2 (Chernoff *et al*., 1979).

In a screening assay for developmental toxicity, 600 mg/kg bw ethylenethiourea were given by oral gavage to 35 CD-1 mice on days 7–14 of gestation, and the growth and viability of the offspring were evaluated after birth for 3 days and compared with those of a group of 45 untreated controls. A significant increase in the frequency of litters that were completely resorbed was found, but there were no effects on postnatal growth or viability (Plasterer *et al*., 1985).

Groups of 20–23 Sprague-Dawley rats were given 0, 15, 25 or 35 mg/kg bw per day ethylenethiourea by oral gavage on gestation days 6–20. There were no signs of maternal toxicity at any dose. The fetal body weights were reduced at the highest dose, which also caused malformations such as cranial meningocoele and meningorrhoea, severe hind limb talipes and short and/or kinky tails. Rats at the two higher doses had higher incidences of dilated brain ventricles and hydroureter than controls (Saillenfait *et al*., 1991).

Six thioureas, including ethylenethiourea, were evaluated for embryotoxicity by injection onto the heart of 3-day-old white Leghorn chicken embryos. The median effective dose of ethylenethiourea for total embryotoxicity (dead and malformed) was 4.5 μmol [460 μg]/egg; it was the least potent of the thioureas tested (Korhonen *et al*., 1982).

Ethylenethiourea was added at a concentration of 20, 30, 40 or 50 μg/mL to cultures of *Daphnia magna* eggs. No eggs hatched at the highest dose, and hatchability was reduced by about 20% at 30 and 40 μg/mL. The incidence of morphological anomalies of the carapace was significantly increased at 20, 30 and 40 μg/mL (Ohta *et al*., 1998).

(*b*) *Phase specificity*

The stage-dependence of the teratogenic effects of ethylenethiourea was demonstrated in Wistar rats exposed by gavage to 40–480 mg/kg bw on one of days 6–21 of gestation. The earliest teratogenic effects were seen after treatment on day 10, and included failure of coccygeal growth, spina bifida, ectopic genitalia and nephrosis. The incidence of defects peaked after exposure on days 12–15, but effects such as hydranencephaly, hydronephrosis and subcutaneous oedema were seen after exposure as late as day 21 (Ruddick & Khera, 1975).

Ethylenethiourea was given orally to Wistar rats at a single dose of 1–50 mg/kg bw in aqueous suspension on day 17, 18, 19 or 20 of gestation. The incidence of stillbirths was increased at doses of 30 and 50 mg/kg bw on day 18, 19 or 20. Regardless of the age at exposure, doses as low as 10 mg/kg bw were associated with reduced offspring viability due to hydrocephaly (Lewerenz & Bleyl, 1980).

Ethylenethiourea has been used as a prototype teratogen to study postnatal functional development of the kidney. Prenatal exposure of Sprague-Dawley rats to

0–160 mg/kg bw ethylenethiourea on day 11 of gestation produced dose-related increases in the incidence of enlarged renal pelvis in the fetuses on day 21 of gestation. Further studies were conducted to explore the postnatal consequences on renal development and function after exposure to 0, 20, 40 or 60 mg/kg bw on day 11 of gestation. The incidence of hydronephrosis after birth was lower than anticipated from the study of prenatal exposure, probably as a result of increased postnatal mortality. The severity of the hydronephrosis, however, increased with postnatal age. The hydonephrotic animals had impaired concentrating ability, but cortical function (proximal tubule transport) was unaffected. Rats exposed to ethylenethiourea prenatally had grossly normal kidneys but showed suppressed electrolyte clearance early in life. The latter effect was no longer apparent by postnatal day 27 (Daston *et al*., 1988).

The effect of prenatal exposure to ethylenethiourea on the development of the posterior gut was studied in 28 Wistar-Imamichi rats treated with 100, 125, 150 or 200 mg/kg bw ethylenethiourea by intragastric administration on day 11 of gestation. Another four pregnant rats were available as controls. Fetuses were examined on day 20 of gestation. The dose-related malformations included absent or kinked tails, spina bifida and myeloschisis. The incidences of malformations were significantly higher in male than female fetuses. Histological examination of 57 fetuses exposed to 125 mg/kg bw revealed an incidence of anorectal malformations in 92% of males and 41% of females (Hirai & Kuwabara, 1990).

The phase specificity of ethylenethiourea was studied in Sprague-Dawley rats exposed by oral gavage to 0, 60, 120 or 240 mg/kg bw on one day of gestation between days 8 and 19. The number of litters per group was not specified, but there were 113 females in the experiment and 717 fetuses (16–86 per group). Fetuses were examined on day 20 for soft-tissue anomalies by histological procedures. A high rate of mortality was seen after exposure on days 8–10. Exposure to the two higher doses resulted in a variety of central nervous system malformations (e.g., spinal raphism, exencephaly, hydranencephaly and hydrocephaly) after exposure on one of days 11–18 of gestation, and the specific malformations showed phase sensitivity. Thus, short tail was observed after exposure on one of days 11–14, spinal raphism after exposure on day 11, exencephaly after exposure on day 12 or 13, microencepahly after exposure on day 14 and hydranencephaly after exposure on day 15 or 16 (Hung *et al.*, 1986). The effects of ethylenethiourea on prenatal brain development were further studied in 20 pregnant Sprague-Dawley rats that were exposed to 60, 120, 240 or 360 mg/kg bw ethylenethiourea by gavage on day 11 of gestation (Hung, 1992). A total of 155 fetuses from the treated groups and 38 fetuses from three controls were examined on day 20 of gestation. Dose-related incidences of malformations, which reached 100% at the highest dose, were observed. The most prominent defects included omphalocoele, lumbosacral myeloschisis and imperforate anus. No malformations were observed in the fetuses of control dams. The author noted that the effects were consistent with an early alteration of mesodermal development (Hung *et al*., 1986).

To study the effect of ethylenethiourea on neural tube development, nine groups of 66 pregnant Long Evans rats received a single intragastric administration of ethylenethiourea on one of days 11–19 of gestation. Each group was further divided into three groups that were given 80, 120 or 160 mg/kg bw ethylenethiourea. A control group of 11 females was available. Fetuses were examined on gestation day 20. Fetal mortality was highest (21%) after treatment on day 11 and was not significantly increased with treatment after day 13. Regardless of the day of treatment, 100% of the fetuses were malformed, except after treatment on day 19, when no malformations were observed. The malformations shifted from myeloschisis with treatment on day 11 to abnormally enlarged head on days 12 and 13 to hydranencephaly and hydrocephalus on days 14–18. Histological examination of the fetuses with myeloschisis indicated hypertrophy of neural tissue, especially in the hindbrain and lower spinal chord. The tissue hypertrophy and rosette formation indicated reparative action in regions of the neural tube where extensive cellular degeneration and necrosis had been reported previously (Sato *et al*., 1985).

(*c*) *Mode of action* in vitro

The direct effect of ethylenethiourea on rodent embryo development was studied in whole-embryo cultures. Addition of 40–200 μg/mL ethylenethiourea to 10-day-old Sprague-Dawley rat embryos and culturing for 48 h *in vitro* resulted in dose-related inhibition of growth and differentiation and increased incidences of malformations. The authors attributed the findings to altered osmotic fluid balance in the embryo, as the osmolality of the exocoelomic fluid was reduced after 48 h in culture (Daston *et al*., 1987).

The development of 10-day-old Sprague-Dawley rat embryos exposed *in vitro* to ethylenethiourea by direct addition of 0–2.0 mmol/L (0–204 μg/mL) ethylenethiourea to the growth medium of whole-embryo culture or exposed *in utero* to 0, 60 or 120 mg/kg bw ethylenethiourea by oral gavage was examined to assess the similarity of the two approaches in inducing central nervous system defects (Khera, 1989). In culture, the embryos showed hydrocephalus after 26 h of exposure to 1.5 or 2.0 mmol/L ethylenethiourea. No hydrocephaly was observed in embryos exposed *in vivo*. The lack of consistency in results obtained *in vitro* and *in vivo* may be due to differences in kinetics and in the critical period of exposure. It has been pointed out that the concentrations and the areas under the curve of concentration–time used *in vitro* are substantially higher than those obtained for teratogenic exposures *in vivo* (Daston, 1990).

The sensitivity of comparably staged Sprague-Dawley rats (gestation day 10.5) and CD-1 mice (gestation day 8.5) in whole-embryo culture was evaluated after a 48-h exposure to ethylenethiourea (at 0, 80, 120 or 160 μg/mL for rats and at 0, 80, 160, 240 or 320 μg/mL for mice). The teratogenic effects were qualitatively similar in the two species and were characterized by excessive accumulation of fluid in structures, particularly in the neural tube, but the potency was approximately twice as great in rats. When an exogenous metabolic activation system from a 9000 × *g* supernatant of liver from

Arochlor 1254-induced rats and mice (S9 mix) was added to the treatment protocol, rat S9 had virtually no effect on the embryonic effects typical of ethylenethiourea, but these were virtually eliminated by mouse S9 in both species. Of note, however, was that addition of mouse S9 and ethylenethiourea to mouse embryos in culture resulted in the induction of abnormalities (mainly open neural tube) not seen in rat or mouse embryos exposed *in vitro* to ethylenethiourea alone, or in mouse embryos exposed *in vivo* (Daston *et al*., 1989).

The effects were studied of direct addition of ethylenethiourea to 11-day-old Wistar-Imamichi rat embryos cultured for 48 h and to midbrain and limb bud cells removed from 11-day-old embryos. Malformations in cultured embryos were observed at concentrations ≥ 30 µg/mL ethylenethiourea. Consistent with the predilection for neural tube defects over limb defects, when the cells were exposed to ethylenethiourea in culture, the median concentration for inhibition of differentiation of midbrain cells was 2.3- and > 14-fold lower than that for limb bud cells on days 11 and 12 of gestation, respectively (Tsuchiya *et al*., 1991a).

Ethylenethiourea was added at 0, 10 or 30 µg/mL to 11.5-day-old Wistar-Imamichi rat embryos in whole-embryo culture for 17 h, and the embryos were then grown in control media for another 29 h. Dose-related morphological anomalies were found that were largely prevented by the addition of an S9 mix from rats induced with phenobarbital and 5,6-benzoflavon (Iwase *et al*., 1997).

Using micromass cultures of midbrain or limb bud cells from 10-day-old JcL/ICR mice or 11-, 12- or 13-day-old Wistar-Imamichi rats exposed either directly to ethylenethiourea in culture (0–600 µg/mL) or using serum of rats and mice treated *in vivo* (collected 2 h after exposure to 200 mg/kg bw), it was demonstrated that the species difference is at least partially intrinsic to the embryo. That is, the concentration of ethylenethiourea required to affect midbrain cell cultures from 10-day-old mouse embryos directly was 11-fold greater than that required for cultures from 12- and 13-day-old rat embryos. In addition, rat, but not mouse, midbrain cell differentiation was affected when serum from treated rats or mice was used in the culture medium. In the rat cell culture, the midbrain was affected more than limb bud cells, in parallel with effects noted in embryos treated *in vivo*. The concentration of ethylenethiourea in rat sera was only twofold higher than that in mouse sera. The study indicates that the species difference is likely to be due to differences in both kinetics and dynamics between rats and mice (Tsuchiya *et al*., 1991b).

(*d*) *Altered thyroid function*

A teratogenic dose (40 mg/kg bw per day) of ethylenethiourea was given by gavage once daily on days 7–15 of gestation to hypothyroid and euthyroid Charles River rats, and the fetuses were examined on day 20 of gestation. Additional euthyroid groups received subcutaneous injections of ethylenethiourea with or without thyroxine (5 µg/0.1 mL per 100 g bw/day) on days 7–15 of gestation. Hypothyroidism was induced by surgical removal of the thyroparathyroid gland at 75 days of age, 3 weeks

before breeding. As expected, the serum concentration of T4 was reduced by this surgery (2.3 versus 6.2 μg/mL). The endogenous concentrations of T4 were further reduced by ethylenethiourea in the thyroparathyroidectomized groups (1.4 versus 2.3 μg/mL, compared with 4.8 versus 5.9 μg/mL in the sham-operated controls). Malformations were present in 100% of the fetuses regardless of thyroid status, although some different malformations (e.g., oedema, micrognathia, cleft palate and micromelia) were seen in the thyroparathyroidectomized females given ethylenethiourea. The only increase in the incidence of malformations in control groups was a 10.3% incidence in the thyroparathyroidectomized animals not treated with ethylenethiourea. The results did not support a role of altered thyroid function in ethylenethiourea-induced teratogenesis in rats (Lu & Staples, 1978).

The teratogenic potential of ethylenethiourea was compared with that of the thyroid antagonist methimazole (see monograph in this volume) in rat embryo cultures. Exposure of 9.5-day-old Wistar rat embryos to ethylenethiourea at a concentration of 50 μmol/L to 1 mmol/L for 48 h resulted in dose-related reductions in embryonic growth and differentiation; the effects were significant at concentrations of 500 μmol/L and 1 mmol/L. The commonest anomaly was abnormal development of the caudal region of the neural tube. While some similarities in embryonic responses were noted, reductions and swellings of the caudal region in many embryos exposed to ethylenethiourea was not seen in embryos exposed to methimazole, and other effects seen in methimazole-exposed embryos were not seen in ethylenethiourea-treated embryos (Stanisstreet *et al.*, 1990).

(*e*) *Other aspects of developmental toxicity*

In order to study the potential of nitrites to activate ethylenethiourea by nitrosation, the teratogenic effects of ethylenethiourea were studied in SLC-ICR mice exposed to either ethylenethiourea alone or ethylenethiourea in combination with sodium nitrite. The authors hypothesized that ethylenethiourea would react with nitrite at the low pH in the stomach and form a reactive *N*-nitroso compound. Ethylenethiourea was administered by oral gavage at 400 mg/kg bw with or without 200 mg/kg bw sodium nitrite on day 6, 8, 10 or 12 of gestation. There were 12–29 dams in each group, and the fetuses were examined on gestation day 18. When nitrite was administered 2 h after treatment with ethylenethiourea, no teratogenic effects were seen in mouse embryos. Concomitant treatment on day 6 was most effective for induction of fetal death and growth retardation, while various malformations were present after exposure on day 6, 8 or 10. Exposure on day 12 did not adversely effect embryonic development. In particular, treatment on day 6 or 8 caused abnormal lobation of the left and right lung, respectively. Some of the observed defects resembled those observed in ethylenethiourea-treated rats (Teramoto *et al.*, 1980).

The role of altered hepatic function in ethylenethiourea-induced teratogenicity was studied in Swiss-Webster mice that received 0, 1600, 2000 or 2400 mg/kg bw ethylenethiourea by oral gavage on day 12 of gestation. The modulating treatments included

phenobarbital (at 60 mg/kg bw per day by subcutaneous injection on days 7–10 of gestation), SKF-525A (at 40 mg/kg bw by intraperitoneal injection on day 12) and 3-methylcholanthrene (at 20 mg/kg bw per day on days 10–12). Ethylenethiourea alone induced dose-related incidences of hindpaw ectrodactyly and syndactyly and low incidences of cleft palate and hindpaw polydactyly. The incidence of defects was altered only by 3-methylcholanthrene, which reduced the incidences of hindpaw ectrodactyly, syndactyly and cleft palate at the two higher doses of ethylenethiourea (Khera, 1984).

Histological changes to the central nervous system were studied after exposure of Wistar rats to 0, 15 or 30 mg/kg bw ethylenethiourea by oral gavage on day 13 of gestation. Four to six females per dose were killed 12, 24, 48 and 72 h after exposure. Other dams were allowed to litter, and their offspring were followed to postnatal day 80. Within 12 h of receiving the higher dose, karyorrhexis was evident in the germinal layer of the basal lamina of the central nervous system, extending from the spinal cord to the telencephalon. By 48 h, rosettes were present in the neuroepithelium and there was extensive disorganization of the germinal and mantle layers. Similar, but less severe responses were seen at the lower dose. During the postnatal phase, 50% of the offspring of dams at the higher dose had died by 80 days after birth, and hydrocephaly was invariably present. There were no postnatal effects at the lower dose (Khera & Tryphonas, 1985).

4.4 Effects on enzyme induction or inhibition and gene expression

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems*

Male WIST rats and male RIEMS/A mice were given oral doses of ethylenethiourea on 3 consecutive days. At a dose of 75 mg/kg bw per day, decreased activities of cytochrome P450 enzymes and aniline hydroxylase were noted in the rats 3 days after treatment. Aminopyrine *N*-demethylase activity was reduced to 60–70% of control values 24 h after treatment with doses of 50 and 75 mg/kg bw per day. In mice, the activity of cytochrome P450 enzymes was increased 24 h after treatment with doses of ethylenethiourea ranging from 50 to 1000 mg/kg bw per day, and aniline hydroxylase activity was increased at doses of 100–1000 mg/kg bw per day. No change in aminopyrine-*N*-demethylase activity was seen (Lewerenz & Plass, 1984).

After a single oral dose of 50–600 mg/kg bw ethylenethiourea, male Swiss mice showed an increase (up to 2.4-fold) in microsomal aniline hydroxylase activity, which returned to control levels within 4 days after treatment. Treatment with actinomycin D, a transcription inhibitor, completely prevented the increase in enzyme activity when given by intraperitoneal injection 1 h before and 5 h after ethylenethiourea (Meneguz & Michalek, 1986).

In a study with microsomes from male and female Swiss-Webster mice, ethylenethiourea was shown to be preferentially metabolized by flavin-dependent monooxygenases, with binding of ethylenethiourea metabolites to liver microsomes (Hui *et al.*, 1988).

4.5 Genetic and related effects

The genotoxicity of ethylenethiourea has been reviewed (Dearfield, 1994; Elia *et al.*, 1995; Houeto *et al.*, 1995).

4.5.1 *Humans*

The frequency of sister chromatid exchange was increased in peripheral lymphocytes of pesticide applicators who had presumably been exposed to ethylenethiourea as a metabolite of ethylenebisthiocarbamate fungicides. In the same study, the exposed individuals also had a higher frequency of chromosomal translocations than controls but not of other types of chromosomal damage (Steenland *et al.*, 1997).

4.5.2 *Experimental systems* (see Table 4 for references)

(*a*) *DNA damage*

Ethylenethiourea did not induce SOS repair in *Salmonella typhimurium* or *Escherichia coli*. It induced λ phage in *Escherichia coli*. It was weakly active in the *E. coli polA* test for differential toxicity only in liquid suspension; it caused differential toxicity in one *E. coli rec* assay and equivocal results in two assays in *Bacillus subtilis rec*.

Ethylenethiourea induced DNA damage in the yeast *Saccharomyces cerevisiae*, as measured by differential survival of repair-deficient strains.

(*b*) *Mutation and allied effects* in vitro

Ethylenethiourea was not mutagenic in *S. typhimurium* with or without metabolic activation, except in a few base-pair substitution or frameshift strains with metabolic activation. No mutation was induced in *E. coli*, except for a weak response in one study. In mouse or rat host-mediated assays, no mutations were induced in *S. typhimurium* G46 or TA1950, but a positive response was seen in *S. typhimurium* TA1530 in mice.

Ethylenethiourea did not induce forward mutation in *Schizosaccharomyces pombe*, but it induced reverse mutation in *Saccharomyces cerevisiae*. It induced mitotic gene conversion in one study but not in others, and induced intrachromosomal recombination and aneuploidy in yeast. Ethylenethiourea marginally induced petite mutants in yeast.

There is disagreement in the literature with regard to the mutagenicity of ethylenethiourea at the *Tk* locus in mouse lymphoma L5178Y cells. It was not mutagenic at multiple loci in Chinese hamster ovary cells with or without S9. Ethylenethiourea did

Table 4 Genetic and related effects of ethylenethiourea

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Escherichia coli, λ phage induction	NT	+	10 000	Thomson (1981)
Escherichia coli, SOS repair (Chromotest), forward mutation	–	–	NR	Quillardet *et al*. (1985)
Salmonella typhimurium, SOS repair (Vitotox) test	–	NT	NR	van der Lelie *et al*. (1997)
Escherichia coli pol A, differential toxicity (liquid suspension test)	(+)	–	NR	Rosenkranz *et al*. (1981)
Escherichia coli pol A, *lex*A, *rec*A, differential toxicity	–	–	NR	Green (1981)
Escherichia coli pol A, *lex*A, *rec*A, differential toxicity	–	–	1000	Tweats (1981)
Escherichia coli rec assay, differential toxicity (spot test)	NT	+	NR	Ichinotsubo *et al*. (1981a)
Bacillus subtilis rec assay, differential toxicity	(+)	–	2 mg/disc	Kada (1981)
Bacillus subtilis rec assay, differential toxicity	–	NT	4 mg/disc	Teramoto *et al*. (1977)
Salmonella typhimurium, forward mutation, *aza* resistance	NT	–	100	Skopek *et al*. (1981)
Salmonella typhimurium TA100, reverse mutation	–	–	20 000 µg/plate	Teramoto *et al*. (1977)
Salmonella typhimurium TA100, TA1535, TA1538, TA98, reverse mutation	NT	+	20 µg/plate	Anderson & Styles (1978)
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	2000 µg/plate	Brooks & Dean (1981); Rowland & Severn (1981)
Salmonella typhimurium TA100, TA98, reverse mutation	–	–	500 µg/plate	Venitt & Crofton-Sleigh (1981)
Salmonella typhimurium TA100, TA98, reverse mutation	–	–	500	Hubbard *et al*. (1981)
Salmonella typhimurium TA100, reverse mutation	–	–	NR	Ichinotsubo *et al*. (1981b)
Salmonella typhimurium TA98, reverse mutation	–	+	NR	Ichinotsubo *et al*. (1981b)
Salmonella typhimurium TA100, TA98, reverse mutation	–	–	5000 µg/plate	MacDonald (1981); Franekic *et al*. (1994)
Salmonella typhimurium TA100, TA1537, TA98, reverse mutation	–	–	NR	Nagao & Takahashi (1981)

Table 4 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	2500 µg/plate	Trueman (1981)
Salmonella typhimurium TA100, TA98, reverse mutation	–	–	100 µg/plate	Kanamaru *et al*. (1984)
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	1000 µg/plate	Falck *et al*. (1985)
Salmonella typhimurium TA100, TA1537, TA98, reverse mutation	–	–	10 000 µg/plate	Mortelmans *et al*. (1986)
Salmonella typhimurium TA102, TA104, reverse mutation	–	–	50 µg/plate	Franekic *et al*. (1994)
Salmonella typhimurium TA1530, reverse mutation	+	NT	40 000 µg/plate	Schüpbach & Hummler (1977)
Salmonella typhimurium TA1535, reverse mutation	+	–	5000 µg/plate	Teramoto *et al*. (1977)
Salmonella typhimurium TA1535, TA1537, TA98, reverse mutation	–	–	1000	Gatehouse (1981)
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	5000 µg/plate	Richold & Jones (1981)
Salmonella typhimurium TA1535, reverse mutation	?	+	1000 µg/plate	Simmon & Shepherd (1981)
Salmonella typhimurium TA1535, reverse mutation	+	+	5000 µg/plate	Moriya *et al*. (1983)
Salmonella typhimurium TA1535, reverse mutation	(+)	(+)	3333 µg/plate	Mortelmans *et al*. (1986)
Salmonella typhimurium TA1537, TA1538, reverse mutation	–	–	10 000 µg/plate	Teramoto *et al*. (1977)
Salmonella typhimurium TA1537, reverse mutation	–	–	2000 µg/plate	MacDonald (1981)
Salmonella typhimurium TA100, TA1537, TA1538, TA98, reverse mutation	–	–	5000 µg/plate	Simmon & Shepherd (1981); Moriya *et al*. (1983)
Salmonella typhimurium TA98, reverse mutation	–	+	100 µg/plate	Garner *et al*. (1981)
Salmonella typhimurium G46, reverse mutation (spot test)	+	NT	100	Seiler (1974)

Table 4 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Salmonella typhimurium G46, reverse mutation	–	NT	80 000 µg/plate	Schüpbach & Hummler (1977)
Salmonella typhimurium G46, reverse mutation	–	–	10 000 µg/plate	Teramoto *et al*. (1977)
Salmonella typhimurium TA1531, TA1532, TA1964, reverse mutation (spot test)	–	NT	NR	Schüpbach & Hummler (1977)
Salmonella typhimurium TA92, reverse mutation	–	–	2000 µg/plate	Brooks & Dean (1981)
Salmonella typhimurium TA1950, reverse mutation	+	NT	10 000 µg/plate	Autio *et al*. (1982)
Salmonella typhimurium TA1950, reverse mutation (spot test)	+	NT	5 mg/disc	Autio *et al*. (1982)
Escherichia coli K-12/343/113, forward or reverse mutation	–	(+)	0.2 mg/mL	Mohn *et al*. (1981)
Escherichia coli WP2 *hcr*, reverse mutation	–	–	10 000 µg/plate	Teramoto *et al*. (1977)
Escherichia coli WP2 *uvr*A, reverse mutation	–	–	1000	Gatehouse (1981); Falck *et al*. (1985)
Escherichia coli WP2 *uvr*A, WP2, reverse mutation	–	–	NR	Matsushima *et al*. (1981)
Escherichia coli WP2 *uvr*A, reverse mutation	–	–	1000 µg/plate	Falck *et al*. (1985)
Escherichia coli WP2 *uvr*A pKM101, reverse mutation	–	–	500 µg/plate	Venitt & Crofton-Sleigh (1981)
Escherichia coli WP2 pKM101, reverse mutation	–	–	100 µg/plate	Venitt & Crofton-Sleigh (1981)
Escherichia coli WP2 *hcr*, reverse mutation	–	–	5000 µg/plate	Moriya *et al*. (1983)
Saccharomyces cerevisiae, repair-deficient strain, differential toxicity	+	+	300	Sharp & Parry (1981a)
Saccharomyces cerevisiae D6, petite mutations	(+)	NT	1000	Wilkie & Gooneskera (1980)
Saccharomyces cerevisiae D4, mitotic gene conversion	–	–	333 µg/plate	Jagannath *et al*. (1981)

Table 4 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Saccharomyces cerevisiae T_1 and T_2, mitotic crossing-over	–	–	1000	Kassinova *et al*. (1981)
Saccharomyces cerevisiae JD1, mitotic gene conversion	+	NT	50	Sharp & Parry (1981b)
Saccharomyces cerevisiae D7, mitotic gene conversion	–	–	2 mg/mL	Zimmermann & Scheel (1981)
Saccharomyces cerevisiae RS112, intrachromosomal recombination	+	NT	20 mg/mL	Schiestl *et al*. (1989)
Saccharomyces cerevisiae D6 (stationary phase cells), mitotic aneuploidy	+	NT	500	Parry & Sharp (1981)
Saccharomyces cerevisiae, chromosome loss	+	NT	400	Franekic *et al*. (1994)
Saccharomyces cerevisiae, reverse mutation	+	–	88.9	Mehta & von Borstel (1981)
Schizosaccharomyces pombe, forward mutation	–	–	1	Loprieno (1981)
Aspergillus nidulans 35, forward mutation	–	NT	11 860	Crebelli *et al*. (1986)
Aspergillus nidulans P_1, mitotic malsegregation	+	NT	4000	Crebelli *et al*. (1986)
Shallot root tips, micronucleus formation	+	NT	2.5	Franekic *et al*. (1994)
Shallot root tips, chromosomal aberrations	+	NT	2.5	Franekic *et al*. (1994)
Drosophila melanogaster, somatic recombination, w/w^+ locus	–		51.1 mg/kg feed	Vogel & Nivard (1993)
Drosophila melanogaster, somatic recombination, w/w^+ locus	+		51.1 mg/kg feed	Rodriguez-Arnaiz (1997)
Drosophila melanogaster, sex-linked recessive lethal mutation	–		250 mg/kg feed	Valencia & Houtchens (1981)
Drosophila melanogaster, sex-linked recessive lethal mutation	–		4900 mg/kg inj	Woodruff *et al*. (1985)
Drosophila melanogaster, sex-linked recessive lethal mutation	–		12 500 mg/kg feed	Woodruff *et al*. (1985)

Table 4 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Drosophila melanogaster, sex-linked recessive lethal mutation	–		5100 mg/kg feed	Mason *et al*. (1992)
Gene mutation, Chinese hamster ovary cells, resistance to 8-azaadenine, 6-thioguanine, ouabain octahydrate, 5-fluoro-deoxyuridine, *in vitro*	–	–	2000	Carver *et al*. (1981)
Gene mutation, *Tk* locus, mouse lymphoma L5178Y cells *in vitro*	–	–	3000	Jotz & Mitchell (1981)
Gene mutation, *Tk* locus, mouse lymphoma L5178Y cells *in vitro*	–	+	1800	McGregor *et al*. (1988)
Sister chromatid exchange, Chinese hamster ovary cells *in vitro*	–	–	1000	Evans & Mitchell (1981)
Sister chromatid exchange, Chinese hamster ovary cells *in vitro*	–	–	5000	Natarajan & van Kesteren-van Leeuwen (1981)
Sister chromatid exchange, Chinese hamster ovary cells *in vitro*	–	–	100	Perry & Thomson (1981)
Sister chromatid exchange, Chinese hamster ovary cells *in vitro*	–	–	5000	National Toxicology Program (1992)
Cell transformation, BALB/c-3T3 mouse cells	(+)	NT	NR	Matthews *et al*. (1993)
Cell transformation, BHK-21 mouse cells	+	+	NR	Daniel & Dehnel (1981)
Cell transformation, BHK-21 mouse cells	+	NT	0.2	Styles (1981)
Cell transformation, SA7/Syrian hamster embryo cells	(+)	NT	1000	Hatch *et al*. (1986)
Micronucleus formation, Syrian hamster embryo cells *in vitro*	–	NT	NR	Fritzenschaf *et al*. (1993)
Chromosomal aberrations, Chinese hamster DON cells *in vitro*	–	NT	3200	Teramoto *et al*. (1977)
Chromosomal aberrations, Chinese hamster ovary cells *in vitro*	?[c]	?[c]	5000	Natarajan & van Kesteren-van Leeuwen (1981)
Chromosomal aberrations, Chinese hamster ovary cells *in vitro*	–	–	10 000	National Toxicology Program (1992)
Chromosomal aberrations, rat liver RL1 cells *in vitro*	–	NT	200	Dean (1981)

Table 4 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Host-mediated assay, *Salmonella typhimurium* G46 in Swiss albino mice	–		6000 mg/kg bw; im × 1	Schüpbach & Hummler (1977)
Host-mediated assay, *Salmonella typhimurium* TA1530 in Swiss albino mice	+		6000 mg/kg bw; im × 1	Schüpbach & Hummler (1977)
Host mediated assay, *Salmonella typhimurium* TA1950 in male NMRI mice	–		5 po × 1	Autio *et al.* (1982)
Host-mediated assay, *Salmonella typhimurium* G46 in JCR-ICR mice and Wistar rats	–		400 po × 3	Teramoto *et al*. (1997)
DNA damage (Comet assay), male CD-1 mouse liver, kidney, lung and spleen *in vivo*	+		2000 ip × 1	Sasaki *et al*. (1997)
DNA damage (Comet assay), male CD-1 mouse bone marrow *in vivo*	–		2000 ip × 1	Sasaki *et al*. (1997)
Sister chromatid exchange, male CBA/J mouse bone-marrow cells *in vivo*	–		1000 ip × 1	Paika *et al*. (1981)
Micronucleus formation, female ICR mouse bone-marrow cells *in vivo*	–		450 ip × 2	Seiler (1973)
Micronucleus formation, mouse bone-marrow cells *in vivo*	–		6000 po × 2	Schüpbach & Hummler (1977)
Micronucleus formation, male ICR mouse bone-marrow cells *in vivo*	–		880 ip × 1	Kirkhart (1981)
Micronucleus formation, $B6C3F_1$ mouse bone-marrow cells *in vivo*	(+)		1400 ip × 2[d]	Salamone *et al*. (1981)
Micronucleus formation, CD-1 mouse bone-marrow cells *in vivo*	–		880 ip × 2	Tsuchimoto & Matter (1981)
Micronucleus formation, CD-1 mouse peripheral blood and bone-marrow cells *in vivo*	–		2500 ip × 2	Morita *et al*. (1997)
Chromosomal aberrations, male and female Wistar rat bone-marrow cells *in vivo*	–		400 po × 2	Teramoto *et al*. (1977)
Dominant lethal mutation, Swiss albino mice *in vivo*	–		3500 po × 1	Schüpbach & Hummler (1977)

Table 4 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Dominant lethal mutation, JCL-ICR mice *in vivo*	–		600 po × 5	Teramoto *et al*. (1977)
Dominant lethal mutation, C3H/HeCr mice *in vivo*	–		150 po × 5	Teramoto *et al*. (1978)
Inhibition of DNA synthesis, mouse testis *in vivo*	–		100 ip × 1	Seiler (1977)
Sperm morphology, (CBA × BALB/c)F_1 mice *in vivo*	–		2000 ip × 5	Topham (1981)
Sperm morphology, B6C3F_1/CRL mice *in vivo*	–		2655 ip × 5	Wyrobek *et al*. (1981)

[a] +, positive; (+), weak positive; –, negative; NT, not tested; ?, inconclusive

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; ip, intraperitoneal; po, oral; im, intramuscular; inj, injection

[c] No dose–response relationship

[d] Dose is 80% of LD_{50} (as reported by Tsuchimoto & Matter, 1981)

not induce chromosomal aberrations or sister chromatid exchange in cultured Chinese hamster cells or a rat liver cell line or micronuclei in Syrian hamster embryo cells.

Ethylenethiourea transformed BHK-21 cells in culture and had weak transforming activity on BALB/c-3T3 cells.

(*c*) *Mutation and allied effects* in vivo

DNA damage, as measured in the Comet assay, was induced in liver, kidney, lung and spleen, but not bone-marrow cells of mice given an intraperitoneal injection of ethylenethiourea.

Chromosomal aberrations were not induced in rat bone-marrow cells after oral administration, and no sister chromatid exchange was induced in mouse bone-marrow cells after intraperitoneal injection. Micronucleus formation was not induced in mouse blood or bone-marrow cells after intraperitoneal or oral administration.

Ethylenethiourea did not induce dominant lethal mutations or sperm abnormalities or inhibit testicular DNA synthesis in male mice.

In *Drosophila melanogaster*, sex-linked recessive lethal mutations were not induced, but somatic recombination was induced at the w/w^+ locus in one of two studies.

Micronuclei and chromosomal aberrations were induced by ethylenethiourea in shallot root tips.

4.6 Mechanistic considerations

Ethylenethiourea is not genotoxic.

The available data indicate that thyroid hormone imbalance plays a role in the development of follicular-cell neoplasia caused by ethylenethiourea in rats and mice.

- Ethylenethiourea is considered not to be genotoxic because of its lack of activity in appropriate tests in bacteria, mammalian cells *in vitro* and mice and rats treated *in vivo*.
- Ethylenethiourea alters thyroid hormone homeostasis in rats treated with doses spanning the range that induced thyroid tumours in this species.
- Ethylenethiourea produces thyroid gland enlargement (goitre) in rats and follicular-cell hypertrophy and hyperplasia in rats and mice. The mechanism is based on interference with thyroid peroxidase.

On the basis of this information, which meets the criteria laid out in the IARC consensus report (Capen *et al.*, 1999), ethylenethiourea would be expected not to be carcinogenic to humans exposed to concentrations that do not lead to alterations in thyroid hormone homeostasis.

The hyperplasia induced by ethylenethiourea in the thyroid gland is diffuse, in analogy with the morphological changes induced by stimulation of thyroid-stimulating hormone, rather than only multifocal, as would be induced by a genotoxic thyroid carcinogen (Hard, 1998). In view of the lack of genotoxicity, the liver tumours in mice

and the benign pituitary tumours in mice were considered not to be produced by a genotoxic mechanism.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Ethylenethiourea is used as a vulcanization accelerator in the rubber industry. It is a degradation product of and an impurity in ethylenebisdithiocarbamate fungicides, and field workers may be exposed to ethylenethiourea while applying these fungicides. The general population may be exposed to low concentrations of residues of ethylenethiourea in foods.

5.2 Human carcinogenicity data

The available data were inadequate to evaluate the carcinogenicity of ethylenethiourea to humans.

5.3 Animal carcinogenicity data

Ethylenethiourea was tested for carcinogenicity by oral administration in two studies in three strains of mice, with perinatal exposure in one study. It was also tested in five studies in rats by oral administration, with perinatal exposure in one study. In mice, it produced thyroid follicular-cell tumours and tumours of the liver and anterior pituitary gland. In rats, it consistently produced thyroid follicular-cell adenomas and carcinomas. Ethylenethiourea did not cause neoplasms in one strain of hamsters.

5.4 Other relevant data

Ethylenethiourea caused thyroid gland enlargement (goitre) in rats and mice as a result of diffuse hypertrophy and hyperplasia of thyroid follicular cells. Administration of ethylenethiourea under bioassay conditions that caused predominantly benign follicular-cell tumours resulted in alteration of thyroid hormone homeostasis, including increased secretion of thyroid-stimulating hormone. The underlying mechanism of the changes induced by ethylenethiourea is interference with the functioning of thyroid peroxidase activity. This is considered to be the basis for its tumorigenic activity in experimental animals.

One retrospective study of pregnancy outcomes in women employed in the manufacture of rubber containing ethylenethiourea showed no exposure-related effects. Ethylenethiourea was teratogenic in rats, but not in mice, hamsters or guinea-pigs. The central nervous system was particularly vulnerable in rats. The available data suggest

that both toxicokinetics and embryo sensitivity are components of the species-specificity of the teratogenicity of ethylenethiourea. Furthermore, effects on thyroid function do not appear to be involved.

Ethylenethiourea was not genotoxic in appropriate tests in bacteria and cultured mammalian cells or in rodents *in vivo*. Ethylenethiourea induced chromosomal recombination and aneuploidy in yeast and cell transformation in mammalian cells.

5.5 Evaluation

There is *inadequate evidence* in humans for the carcinogenicity of ethylenethiourea.

There is *sufficient evidence* in experimental animals for the carcinogenicity of ethylenethiourea.

Overall evaluation

Ethylenethiourea is not classifiable as to its carcinogenicity to humans (Group 3).

In making its evaluation, the Working Group concluded that ethylenethiourea produces thyroid tumours in mice and rats by a non-genotoxic mechanism, which involves interference with the functioning of thyroid peroxidase resulting in a reduction in circulating thyroid hormone concentrations and increased secretion of thyroid-stimulating hormone. Consequently, ethylenethiourea would not be expected to produce thyroid cancer in humans exposed to concentrations that do not alter thyroid hormone homeostasis.

An additional consideration of the Working Group, based on the lack of genotoxicity of ethylenethiourea, was that the liver tumours and benign pituitary tumours in mice were also produced by a non-genotoxic mechanism.

Evidence from epidemiological studies and from toxicological studies in experimental animals provide compelling evidence that rodents are substantially more sensitive than humans to the development of thyroid tumours in response to thyroid hormone imbalance.

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