4. Mechanistic and Other Relevant Data

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

No data were available to the Working Group.

4.1.2 *Experimental systems*

Data on the absorption, distribution, metabolism and excretion of ethyl carbamate in experimental animals have been reviewed (National Toxicology Program, 2004). Ethyl carbamate is rapidly distributed in body water after administration; it accumulates somewhat more slowly in adipose tissue than in other organs. Earlier studies with labelled ethyl carbamate indicated that it was largely oxidized to carbon dioxide. Its metabolism was suggested to proceed via an esterase reaction that released ethanol, carbon dioxide and ammonia. The rate of elimination was reported to be lower in newborn than in adult mice, which was attributed to the lack of microsomal esterase.

Human CYP2E1 was shown to be a major catalyst of the oxidation of both ethyl carbamate and vinyl carbamate in experiments with human liver microsomes (Guengerich & Kim, 1991; Guengerich *et al.*, 1991). Furthermore, when human liver microsomes were incubated with nicotinamide adenine dinucleotide phosphate (NADPH) and ethyl carbamate, the products vinyl carbamate, 2-hydroxyethyl carbamate and ethyl *N*-hydroxycarbamate were detected (Guengerich & Kim, 1991). The formation of 1,N^6-ethenoadenosine from adenosine in the presence of ethyl carbamate and vinyl carbamate was demonstrated in these studies and it was noted that this reaction was considerably slower with ethyl carbamate. In a separate study, Forkert *et al.* (2001) showed that the metabolism of vinyl carbamate in human lung microsomes is mediated by lung microsomal CYP2E1. Together, these studies suggest that, in human liver, ethyl carbamate can be converted to its proximate DNA-reactive metabolites, a mechanism similar to that suggested to play a role in carcinogenesis in rodents. [The Working

Group noted that (i) experimental evidence suggests great similarities between rodents and humans in the metabolic activation pathways of ethyl carbamate in target tissues (liver and lung); and (ii) the formation of the same proximate carcinogens that are DNA-reactive and thought to play a major role in ethyl carbamate-induced carcinogenesis in rodents probably also occurs in human cells.]

Ethyl carbamate is metabolized by CYP2E1. *N*-Hydroxylation products have carcinogenic properties, but are less potent than ethyl carbamate itself, and *N*-hydroxyethyl carbamate can be converted to ethyl carbamate (Dahl *et al.*, 1978, 1980; National Toxicology Program, 2004). *N*-Hydroxy derivatives are excreted in the urine as glucuronide and other conjugates. Oxidation of ethyl carbamate to vinyl carbamate, and thence to vinyl carbamate epoxide is thought to account for its carcinogenic properties (National Toxicology Program, 2004).

Yamamoto *et al.* (1988) reported that co-administration of ethanol with ethyl carbamate resulted in delayed clearance of ethyl carbamate and its metabolism to carbon dioxide in male mice; ethanol inhibited the metabolism of ethyl carbamate by liver homogenates. Carlson (1994) also found that ethanol inhibited the metabolism of ethyl carbamate, and that the CYP2E1 inhibitor, diethyldithiocarbamate, substantially reduced the metabolism of ethyl carbamate to carbon dioxide in rats.

Hoffler *et al.* (2003) examined the metabolism of ethyl carbamate in *CYP2E1*-knockout mice and in mice that had been treated with the CYP inhibitor, 1-aminobenzotriazole, and concluded that 96% of the metabolism of radiolabelled ethyl carbamate was mediated by CYP2E1. 1-Aminobenzotriazole also markedly inhibited the metabolism of ethyl carbamate in wild-type mice, and inhibited the residual metabolism in knockout mice. It was suggested that both the oxidation of ethyl carbamate to vinyl carbamate and the subsequent generation of the epoxide are catalyzed by CYP2E1.

Hoffler *et al.* (2005) studied the effects of administration of ethyl carbamate to *CYP2E1*-knockout mice for 6 weeks. The appearance of micronucleated erythrocytes was reduced in the knockout mice. Cell proliferation demonstrated by the appearance of K_i-67, was increased in the lung and liver of ethyl carbamate-treated wild-type mice, but not in the knockout animals. It was concluded that metabolism of ethyl carbamate via CYP2E1 was required for its genotoxicity.

These reports suggest that there are important interactions between ethanol and ethyl carbamate. The ability of ethanol to inhibit the clearance of ethyl carbamate suggests that it does so by competing for metabolic conversion by CYP2E1. Since chronic use of ethanol induces CYP2E1, prior chronic ethanol consumption could be predicted to increase the carcinogenicity of ethyl carbamate (as reported for mice treated with ethanol for 3 days; National Toxicology Program, 2004). Simultaneous exposure to ethanol and ethyl carbamate was reported in several studies to reduce the carcinogenicity of the latter (National Toxicology Program, 2004). However, in a 2-year toxicity study (National Toxicology Program, 2004), there was only a weak interaction between ethanol (0, 2.5 and 5% ethanol) and ethyl carbamate when the two compounds

were co-administered *ad libitum* in the drinking-water to mice (see Section 4.4.2(*b*)(i) of the monograph on Alcoholic beverage consumption.).

4.2 Toxic effects

4.2.1 *Humans*

A clinical trial of ethyl carbamate in patients with leukaemia (32 cases) and other types of somatic cancer (13 cases) involved oral administration of doses of 1–6 g per day for 5 to 109 days (Paterson *et al.*, 1946). The total dose varied by patient from 26 to 390 g. Nausea, vomiting and diarrhoea were reported as common side-ffects. Leukopoenia was observed in patients with somatic tumours, while the observed sharp fall in white cell counts was considered to be a beneficial effect in patients with leukaemia. These health effects were reversible when treatment with ethyl carbamate was discontinued. Similar side-effects were observed by Hirschboeck *et al.* (1948) in patients who took 0.5–2 g ethyl carbamate orally in capsules. When administered intramuscularly (2–4 mL of a 50% solution [1–2 g]), dizziness and drowsiness were also reported. No reports of the possible adverse health effects of ethyl carbamate when it was used as a co-solvent in Japanese patients (doses estimated to be 10–50 mg/kg bw; Nomura, 1975a) are available.

4.2.2 *Experimental systems*

Ethyl carbamate is known to induce acute toxic reactions in rodents. In female C57BL/6J mice that received subcutaneous injections of 4000 mg/kg bw ethyl carbamate for 12 days, spleen and thymus weights and circulating leukocyte levels were reduced (Luebke *et al.*, 1987). The immunocompetence of treated mice was also severely compromised, as measured by the delayed hypersensitivity reaction.

Female B6C3F$_1$ mice that received a total dose of 4000 mg/kg bw ethyl carbamate by intraperitoneal injection over 14 days also had lower spleen and thymus weights than the controls, but peripheral blood cell counts were not affected (Luster *et al.*, 1982). The presence of micronuclei in peripheral blood cells of mice following administration of ethyl carbamate supports the possibility that blood-forming organs are targets for the toxicity of ethyl carbamate (Bruce & Heddle, 1979). The hypnotic and anaesthetic properties of ethyl carbamate suggest neuropharmacological effects, which may become significant when the chemical is co-administered with ethanol (Salmon & Zeise, 1991).

Various toxic effects were reported in studies of ethyl carbamate administered for 13 weeks in the drinking-water or in 5% ethanol to rats and mice (National Toxicology Program, 1996). Increased lethality was observed in rats that received more than ~300 mg/kg bw ethyl carbamate. Ethyl carbamate was much more toxic in mice; all mice that received more than 1000 mg/kg bw and many that were given ~300 mg/kg died

before the end of the study. Animals in the high-dose groups had lower body weights, reduced water consumption and exhibited thinness, abnormal posture and ruffled fur. Leukopoenia (primarily lymphocytopoenia) was also observed in rats and mice that received doses of ethyl carbamate of ~20 mg/kg bw and ~300 mg/kg bw, respectively.

In separate 4-week and 2-year studies in which male and female B6C3F$_1$ mice were administered 10–90 mg/kg bw ethyl carbamate in the drinking-water or in 5% ethanol (National Toxicology Program, 2004), no adverse effects on body weight or water consumption were noted at 4 weeks, but increased lethality and decreases in body weight were observed in high-dose groups in the 2-year study.

In a study of ethyl carbamate in the drinking-water conducted by Inai *et al.* (1991), survival of male B6C3F$_1$ mice exposed to 100 mg/kg bw ethyl carbamate for 70 weeks was decreased, but not that of mice exposed to less than 10 mg/kg bw. A similar decrease in survival of NMRI mice exposed to concentrations of up to 12.5 mg/kg bw ethyl carbamate per day in the drinking-water began at approximately 85 weeks into the study (Schmähl *et al.*, 1977).

Acute oral administration of 1000 mg/kg bw ethyl carbamate in water to Swiss albino mice led to loss of consciousness for up to 5 hours (Abraham *et al.*, 1998).

Atrophy of the spleen and thymus was reported in BALB/c mice that received intraperitoneal injections of 200 and 400 mg/kg bw ethyl carbamate for 7 days (Cha *et al.*, 2000, 2001).

4.3 Reproductive toxicity and teratogenicity

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

(a) Teratogenic effects

(i) *Prenatal and transplacental (gestational) exposures*

Takaori *et al.* (1966) investigated the teratogenic response of Wistar rats to 1000 mg/kg bw ethyl carbamate given orally at different times during gestation: during 7 successive days of the first, second or third trimester, on 2 successive days during organogenesis or as a single dose on the 8th or 9th day of gestation. Fetal body weight was decreased in all treated groups compared with that of controls. The mean number of resorbed fetuses was increased in the animals treated during the first and second trimesters; a smaller increase occurred in animals treated during the third trimester. No gross malformations were apparent in the fetuses of dams treated in either the first or third trimester, but dams treated on days 8–13 of gestation produced offspring without tails and one with exencephaly. Offspring of animals treated during either trimester had increased incidences of skeletal malformations, which were most pronounced

when treatment occurred during days 6–12. In rats treated with two consecutive doses of ethyl carbamate (1000 mg/kg bw), similar observations were reported. The most pronounced effects were a decrease in placental weight, a decrease in the number of live fetuses, an increase in the number of resorbed fetuses and an increased incidence of skeletal malformations.

Ferm and Hanover (1966) injected ethyl carbamate once intraperitoneally or intravenously into female hamsters on gestation day 8 and the fetuses were taken 1–3 days later. An intravenous dose of 200 mg/kg bw led to abnormalities in 33% of the fetuses examined. Higher doses of 400, 800 or 1200 mg/kg bw given by either route produced fetotoxicity, as well as fetal abnormalities. The malformations reported were exencephaly, *spina bifida*, convoluted cardiac tubes, non-closing of neural folds and marked degeneration of the anterior neural tube.

Single intraperitoneal doses of 500–3000 mg/kg bw ethyl carbamate were injected into pregnant Syrian hamsters on day 8 of gestation, and fetuses were examined for malformations on day 13 of gestation (DiPaolo & Elis, 1967). Ethyl carbamate was lethal to pregnant dams at the 3000-mg/kg bw dose. At lower doses, a dose-dependent increase in the number of dead or resorbed fetuses was observed. Malformations (exencephaly, microcephaly, encephalocele) were detected in up to 10% of fetuses, although no dose-dependent effect was found.

Sinclair (1950) observed that female mice became infertile when injected subcutaneously with ethyl carbamate at a dose of 1500 mg/kg bw. Injection of 750 mg/kg bw ethyl carbamate into pregnant mice on day 7 of gestation caused abortions and lethal central nervous system defects in fetuses. Failure of the brain to close and degeneration of the brain and spinal cord were also seen in fetuses produced by mothers that were treated with the same dose on day 8 of gestation.

Nishimura and Kuginuki (1958) reported that intraperitoneal injection of 1500 mg/kg bw ethyl carbamate into pregnant mice during gestation days 3–9 led to fetal toxicity, but not malformations. Injection on days 7–8 caused resorption of all fetuses. After injection on days 9–12, fetal malformations (short tails and skeletal malformations) were found.

A single injection of 1500 mg/kg bw ethyl carbamate to CBA and C3HeB mice on day 8.5 of gestation induced exencephaly in both CBA and C3HeB fetuses, although marked strain differences were noted (Tutikawa & Harada, 1972).

Fetal malformations developed in the offspring of female ICR/Jcl mice administered ethyl carbamate by subcutaneous injection as early as day 5 of gestation with a high dose of 1500 mg/kg bw and on day 10 with lower doses of 500–1000 mg/kg bw (Nomura, 1974). An increased incidence of preimplantation loss and of early and late deaths was also reported in this study, but only with the high dose (1500 mg/kg bw) of ethyl carbamate.

Subcutaneous administration of 1000 mg/kg bw ethyl carbamate to pregnant ICR/Jcl mice on day 17 of gestation caused embryonic deaths and malformations (skeletal defects and cleft palate) in the offspring (Nomura, 1975b). Three subcutaneous

injections of 150 mg/kg bw ethyl carbamate to pregnant ICR/Jcl mice on days 9, 10 and 11 led to a significant increase in fetal malformations (Nomura, 1975a).

Nomura (1977) gave a single subcutaneous injection of 1000 mg/kg bw ethyl carbamate to pregnant ICR/Jcl mice on day 9, 10, or 11 of gestation. Cleft palates were the only anomaly seen in the offspring of animals treated on day 9. Polydactyly, cleft palates, tail anomalies and open eyelids were seen after treatment on day 10. Syndactyly, tail anomalies and cleft palates occurred after treatment on day 11. In a separate study, a single subcutaneous injection of 1000 mg/kg bw ethyl carbamate to pregnant ICR/Jcl mice on gestational day 10 led to fetal malformations such as cleft palates, tail anomalies and polydactyly (Nomura, 1983).

Nakane and Kameyama (1986) studied the teratogenicity of ethyl carbamate in CL/Fr mice, a strain that is characterized by a 30% incidence of spontaneous cleft lip with associated cleft palate in the offspring. Pregnant CL/Fr mice were treated with various doses of ethyl carbamate on different days of pregnancy. In the groups treated with 250, 500 and 750 mg/kg bw ethyl carbamate on day 9 of pregnancy, the frequency of cleft lip/palate decreased to 18%, 14% and 11% of term fetuses, respectively. In the group treated with 1000 mg/kg bw ethyl carbamate on day 9, the frequency of cleft lip/palate decreased to 6%, but isolated cleft palate was observed in 23% of term fetuses. Most fetuses in the same group had severe tail anomalies and showed marked loss in body weight.

Treatment of NMRI mice with a single intraperitoneal injection of 800 mg/kg bw ethyl carbamate on day 14 of gestation caused an increased incidence of polydactylism, cleft palate and microphthalmia in fetuses (Burkhard & Fritz-Niggli, 1987).

Treatment of ICR mice with a single subcutaneous injection of 1500 mg/kg bw ethyl carbamate on gestation day 10 resulted in cleft palate in approximately two-thirds of fetuses evaluated at gestation day 14 (Sharova *et al.*, 2003). The fetal weight:placental weight ratio was not changed by treatment with ethyl carbamate in this study; however, treatment resulted in lower weight of both clefted and morphologically normal fetuses.

(ii) *Parental exposures*

Maternal exposures

Nomura (1975b) observed that, when female ICR/Jcl mice received 1500 mg/kg bw ethyl carbamate and were subsequently mated with untreated males at 1–10-week intervals, dominant lethality was higher than that in controls at 2–3-week intervals. Malformed fetuses (open eyelids, kinky tails, cleft palates and dwarfism) were observed at a significantly higher incidence than in controls, and a higher incidence of malformations was observed in the offspring of ethyl carbamate-exposed females than in those of ethyl carbamate-exposed males.

Nomura (1982) administered a single subcutaneous injection of 1000 or 1500 mg/kg bw ethyl carbamate to female ICR mice; the mice were subsequently mated with untreated males (9 weeks). A significant increase in developmental anomalies was

detected in both 19-day-old fetuses and 7-day-old offspring at both doses with no clear dose–response.

In a subsequent study, Nomura (1988) reported a single subcutaneous injection of 1000–2000 mg/kg bw ethyl carbamate to female mice led to a dose-dependent increase in the incidence of phenotypic anomalies (cleft palate, dwarfism, tail anomalies, open eyelid) in the progeny from subsequent matings. It was noted that immature oocytes of 21-day-old females (mated 10 weeks after exposure) were more sensitive than mature oocytes, but no differences were observed in the anomalies detected after birth.

Paternal exposures

Jackson *et al.* (1959) injected male Wistar rats intraperitoneally with five daily doses of 250 mg/kg bw ethyl carbamate and reported no reduction in litter size following mating with unexposed females for up to 6 weeks after treatment.

Bateman (1967) injected male mice intraperitoneally with 1500 mg/kg bw ethyl carbamate and allowed them to mate with unexposed females. Females in the cage were changed each week for up to 9 weeks after treatment of the males. No significant effect on the number of implants, or early or late deaths was observed at any of the time-points. The study also attempted to increase exposure to ethyl carbamate through injections of 1500 mg/kg bw on 3 successive days. However, most males did not survive beyond 2 weeks after treatment. Nevertheless, no significant effect on the number of implants or early or late deaths was observed in embryos from pregnancies that occurred up to 3 weeks after treatment of the males.

Kennedy *et al.* (1973) administered a single intraperitoneal injection of 50 or 100 mg/kg bw ethyl carbamate to male mice and mated them with untreated virgin females that were changed weekly for 6 weeks. Females were sacrificed 1 week after removal from the breeding cage, and their uterine contents were evaluated for numbers of embryos, implantations and resorptions (early and late). The authors reported that genetic damage, as manifested by dominant lethal mutations, did not occur.

Nomura (1975b) administered a single subcutaneous injection of 1500 mg/kg bw ethyl carbamate to male mice, 9 weeks of age, and subsequently mated the mice with untreated females (9 weeks). Dominant lethality was significantly different from that in controls at all experimental stages. A significantly higher incidence of malformed fetuses (open eyelids, kinky tails, cleft palates and dwarfism) was observed after treatment than in controls.

Nomura (1982) administered a single subcutaneous injection of 1500–2000 mg/kg bw ethyl carbamate to male ICR mice and subsequently mated the mice with untreated females (9 weeks). No dominant lethality was detected at any stage of embryonic development. A significant increase in developmental anomalies was detected in both 19-day-old fetuses and 7-day-old offspring after both doses with no clear dose–response.

Russell *et al.* (1987) administered a single intraperitoneal injection of 1750 mg/kg bw ethyl carbamate to male (101 × C3H)F_1 mice that were then mated with unexposed females. Litter sizes from successive conceptions made in any of the first 7 weeks gave no indication of induced dominant lethality.

Nomura (1988) reported that paternal exposure to a single subcutaneous injection of 1000–2000 mg/kg bw ethyl carbamate led to a nonlinear dose-dependent increase in the incidence of phenotypic anomalies (cleft palate, dwarfism, tail anomalies, open eyelid) in F_1 progeny. It was noted that anomalies were induced more effectively in the F_1 fetuses by treatment at the stage of spermatozoa rather than at that of spermatogonia.

Edwards *et al.* (1999) treated male CD-1 mice with ethyl carbamate, either acutely by intraperitoneal injection of 1250 and 1750 mg/kg bw, or subchronically in the drinking-water at 190 mg/kg bw for 10 weeks and 370 mg/kg bw for 9 weeks. One week after the end of each treatment, male mice were mated with untreated females. No genetic effect of acute treatment with ethyl carbamate on male germ cells, as indicated by dominant lethality, was observed. No skeletal or other malformations were observed following acute paternal exposure. A significant increase in post-implantation deaths was observed only after acute administration of ethyl carbamate (1750 mg/kg) and the authors suggested that this was possibly due to perinatal mortality, since no such increase occurred in the dominant lethal part of the study. No effects were observed in offspring of males treated subchronically with ethyl carbamate in the drinking-water.

(iii) *Postnatal exposures*

Increased tumour incidence is the most frequently reported effect of perinatal exposure to ethyl carbamate. These studies are described in detail in Section 3.

(b) Effects on male and female reproductive systems

Russell *et al.* (1987) administered a single intraperitoneal injection of 1750 mg/kg bw ethyl carbamate to male (101 × C3H)F_1 mice. Cytotoxic effects on male reproduction were evident from a slight reduction in the numbers of certain types of spermatogonia in seminiferous tubule cross-sections and a borderline decrease in the number of litters conceived during the 8th and 9th weeks after treatment.

Nomura (1988) reported that a single subcutaneous injection of 1000 or 1500 mg/kg bw ethyl carbamate to male mice did not decrease their fertility for up to 180 days after exposure.

Yu *et al.* (1999) reported no significant decreases in the total number of litters or the average number of offspring born per litter when male NIH Swiss mice were exposed intraperitoneally to 1500 mg/kg bw ethyl carbamate and mated with unexposed females 2 weeks later.

A 13-week study of ethyl carbamate administered in the drinking-water to Fischer 344/N rats (National Toxicology Program, 1996) reported that the only parameter affected in the reproductive system in males was lowered epididymal spermatozoal motility and concentration in the 78- and 287-mg/kg bw groups. When ethyl carbamate was administered in a 5% ethanol vehicle, the responses were similar to those with the drinking-water vehicle. The length of the estrous cycle of female rats that received 201 mg/kg bw ethyl carbamate in 5% ethanol was longer than that of the controls. This

effect was not observed when ethyl carbamate was added to the drinking-water at a dose of 332 mg/kg bw, but was observed with a dose of 525 mg/kg bw.

A 13-week study of ethyl carbamate administered in the drinking-water to B6C3F$_1$ mice (National Toxicology Program, 1996) reported that minimal to mild degeneration occurred in the testes of males administered ~1500 mg/kg bw. Degeneration of the seminiferous tubules, characterized by loss of germ cells and the presence of a few to numerous spermatid giant cells within tubule lumens, was observed in five males that received ~1500 mg/kg bw. The histopathological changes in the testis were considered to be secondary to the debilitated condition of the mice, as these changes occurred only in mice that died early. Epididymal spermatozoal concentration was generally lower in exposed males than in the controls, and the difference was significant in the 30- and 191-mg/kg bw groups. Spermatozoal motility was also lower in males in the 191-mg/kg bw group than in controls. In females, minimal to mild degeneration occurred in the ovaries at doses above 1500 mg/kg bw. The degenerative changes in the ovarian follicles consisted of greater amounts of cell debris within developing follicles than that observed in control females. The histopathological changes in the ovaries were considered to be secondary to the debilitated condition of the mice, as these changes occurred only in mice that died early. In seven females in the 511-mg/kg bw group, the ovaries were smaller than those of the controls as a result of decreased numbers of follicles and *corpora lutea* and the flattening of interstitial cells and females in this group had effectively ceased to have an estrous cycle. In nine females, no cyclicity was demonstrated, while in the remaining female, the percentage of diestrous smears was doubled.

In the same study (National Toxicology Program, 1996), when ethyl carbamate was administered in 5% ethanol, the effects on epididymal spermatozoal concentration in male mice did not appear to be enhanced. Spermatozoal motility was lower in males in the 370-mg/kg bw group. It was noted that, if 5% ethanol had any effect on the toxicity of ethyl carbamate in the male reproductive system in mice, this may have been masked due to the lower fluid (and therefore ethyl carbamate) consumption in that study. In females, the 5% ethanol vehicle appeared to enhance ethyl carbamate-induced ovarian atrophy. Other effects produced with the water vehicle were also observed when 5% ethanol was used as a vehicle.

Non-neoplastic lesions of the reproductive system in female B6C3F$_1$ mice were assessed in a 2-year study (National Toxicology Program, 2004). In the uterus of females exposed to increasing concentrations of ethyl carbamate in drinking-water that contained 0% or 2.5% ethanol, the incidence of angiectasis (dilated vascular spaces lined by a single layer of essentially normal endothelial cells) and thrombosis had a positive trend, and was significantly increased in females exposed to ~3 and 12 mg/kg bw ethyl carbamate. In female mice that received ethyl carbamate in 5% ethanol vehicle, no significant effect on these parameters was observed. Haemorrhage from large areas of uterine angiectasis was the cause of death in five females (one exposed to ~3 mg/kg bw and four exposed to ~10 mg/kg bw ethyl carbamate). No significant effects of ethyl carbamate on the male reproductive system were reported in this study.

In the study by Edwards *et al.* (1999), some of the male mice treated acutely with an intraperitoneal injection of 1750 mg/kg bw ethyl carbamate exhibited partial infertility; however, none of the mice treated with 1250 mg/kg bw had adverse effects on reproductive ability. Similarly, no effects on fertility were noted when male mice were treated with ethyl carbamate in the drinking-water at 190 mg/kg bw for 10 weeks or 370 mg/kg bw for 9 weeks.

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems (see Table 4.1 for details and references)*

Ethyl carbamate is a weak mutagen in prokaryotes (*Bacillus subtilis, Escherichia coli* and *Salmonella typhimurium*). It appears to be a weak mutagen in fungi, and its mutagenicity and genotoxicity vary greatly in different tester strains. Ethyl carbamate is clearly mutagenic *in vivo* in *Drosophila* and induces sex-linked recessive lethal mutations and reciprocal translocations in germ cells.

The results of in-vitro clastogenicity tests with ethyl carbamate in mammalian systems vary among assays; the infrequent positive responses appeared most often with high doses and with exogenous metabolic activation in specific cell types under stringent conditions. Most of the data indicate that ethyl carbamate is inefficient in causing point mutations in mammalian cells *in vitro*.

A limited number of studies was performed to assess the clastogenicity of ethyl carbamate in human cells *in vitro*, and showed that ethyl carbamate induces sister chromatid exchange in human lymphocytes and causes DNA damage (measured as unscheduled DNA synthesis) in human fibroblasts *in vitro*. However, it was reported that ethyl carbamate does not induce micronucleus formation in human lymphocytes or cause chromosomal aberrations in human germ cells *in vitro*. Furthermore, no effect of ethyl carbamate on gene mutations was observed in a human lymphoblastoid cell line.

Results from in-vivo somatic cell assays with ethyl carbamate in mammalian species were generally positive. Chromosomal aberrations, sister chromatid exchange, gene mutation, DNA damage and micronucleus formation were induced with a wide range of doses and in a large number of experimental model organisms (mice, rats and hamsters) and tissues (liver, bone marrow and lungs). Classical clastogenic effects such as chromosomal aberrations were less dose-dependent than sister chromatid exchange. In studies that also assessed the ability of ethyl carbamate to induce cancer, a poor correlation was found between its carcinogenicity and clastogenicity. Ethyl carbamate also induced point mutations in somatic cells *in vivo*.

Table 4.1 Genetic and related effects of ethyl carbamate

Test system	Result[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Bacillus subtillis, M45 *rec*-, differential toxicity	(+)[c]	(+)[c]	2000	Ashby & Kilbey (1981)
Escherichia coli polA-, differential toxicity	–	–	2500	Ashby & Kilbey (1981)
Escherichia coli WP2-WP100, differential toxicity	NT	–	2000	Mamber *et al*. (1983)
Escherichia coli recA-, differential toxicity	(+)[d]	(+)[d]	2000	Ashby & Kilbey (1981)
Escherichia coli gal operon, reverse mutation	NT	–	2000	Ashby & Kilbey (1981)
Escherichia coli PQ37 SOS, reverse mutation	–	–	1000	Dayan *et al*. (1987)
Escherichia coli WP2 *uvrA*, reverse mutation	NT	(+)[e]	25	Bridges *et al*. (1981)
Escherichia coli WP2 *uvrA*, reverse mutation	–	NT	5340	Pai *et al*. (1985)
Escherichia coli K12 *uvrB/recA*, DNA repair host-mediated assay	–	–	50163	Hellmér & Bolcsfoldi (1992)
Salmonella typhimurium TA100, TA1535, TA1537, TA98, reverse mutation	–	–	10000	McCann *et al*. (1975)
Salmonella typhimurium TA100, TA1535, reverse mutation	–	–	400	Dahl *et al*. (1978)
Salmonella typhimurium TA100, TA1535, TA1536, TA1537, TA98, reverse mutation	–	–	125	Simmon (1979a)
Salmonella typhimurium TA100, reverse mutation	–	–	13	Dahl *et al*. (1980)
Salmonella typhimurium TA100, reverse mutation	NT	(+)[e]	25	Bridges *et al*. (1981)
Salmonella typhimurium TA100, TA1537, TA98, TA97, reverse mutation	–	–	10000	National Toxicology Program (1996)
Salmonella typhimurium TA100, TA102, TA98, reverse mutation	–	+	5000	Hübner *et al*. (1997)

Table 4.1 (continued)

Test system	Result[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Salmonella typhimurium TA1535, reverse mutation	–	+	6666	National Toxicology Program (1996)
Salmonella typhimurium YG7108pin3ERb5, reverse mutation	–	NT	10000	Emmert *et al.* (2006)
Salmonella typhimurium TA98, frame shift mutation	–	–	400	Dahl *et al.* (1978)
Salmonella typhimurium TA98, frame shift mutation	NT	(+)[c]	25	Bridges *et al.* (1981)
Salmonella typhimurium TA98, frame shift mutation	(+)[f]	NT	500	Flückiger-Isler *et al.* (2004)
Salmonella typhimurium RS112, DEL recombination	+	+	20000	Galli & Schiestl (1998)
Saccharomyces cerevisiae D3, mitotic recombination	–	–	50000	Simmon (1979b)
Saccharomyces cerevisiae D4, mitotic recombination	–	–	333 μg/plate	Jagannath *et al.* (1981)
Saccharomyces cerevisiae T1, T2, mitotic recombination	–	–	1000	Kassinova *et al.* (1981)
Saccharomyces cerevisiae JD1, mitotic recombination	+	+	150	Sharp & Parry (1981)
Saccharomyces cerevisiae D7, mitotic recombination	–	–	4800	Zimmermann & Scheel (1981)
Saccharomyces cerevisiae XV185-14C, reversion	–	(+)	889	Mehta & von Borstel (1981)
Saccharomyces cerevisiae D6, aneuploidy	–	–	600	Parry & Sharp (1981)
Saccharomyces cerevisiae YB110, chromosomal translocation	+	NT	75000	Hübner *et al.* (1997)
Schizosaccharamyces pombe P1, forward mutation	–	–	4.6	Loprieno (1981)
Aspergillus nidulans, aneuploidy	+	NT	20000	Crebelli *et al.* (1986)
Aspergillus nidulans, forward mutation	–	NT	40000	Crebelli *et al.* (1986)
Neurospora crassa, aneuploidy	–	NT	100	Griffiths *et al.* (1986)
Drosophila melanogaster, sex-linked recessive lethal mutation	+		267000	Vogt (1948)
Drosophila melanogaster, sex-linked recessive lethal mutation	+		222750	Oster (1958)

Table 4.1 (continued)

Test system	Result[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Drosophila melanogaster, sex-linked recessive lethal mutation	+		10000	Knaap & Kramers (1982)
Drosophila melanogaster, somatic mutation and recombination	+		445	Frölich & Würgler (1990)
Drosophila melanogaster, somatic mutation and recombination	+		1800	Graf & van Schaik (1992)
Drosophila melanogaster, somatic mutation and recombination	+		890	Osaba *et al*. (1999)
Drosophila melanogaster, somatic mutation and recombination	+		445	Dogan *et al*. (2005)
Drosophila melanogaster, genetic crossing-over or recombination	+		225	Nivard & Vogel (1999)
DNA strand breaks, rat hepatocytes *in vitro*	+	NT	8900	Sina *et al*. (1983)
Unscheduled DNA synthesis, Holtzman rat hepatocytes *in vitro*	–	NT	890	Sirica *et al*. (1980)
Gene mutation, mouse lymphoma L5178Y cells, *Tk* locus *in vitro*	–	–	3000	Jotz & Mitchell (1981)
Gene mutation, mouse lymphoma L5178Y cells, *Tk* locus *in vitro*	NT	–	11000	Amacher & Turner (1982)
Gene mutation, mouse lymphoma L5178Y cells, *Tk* locus *in vitro*	–	–	5000	Sofuni *et al*. (1996)
Sister chromatid exchange, Chinese hamster DON cells *in vitro*	+	NT	890	Abe & Sasaki (1977)
Sister chromatid exchange, Chinese hamster lung V79 cells *in vitro*	–	–	25	Popescu *et al*. (1977)
Sister chromatid exchange, Chinese hamster lung V79 cells *in vitro*	–	–	20000	Allen *et al*. (1982)
Sister chromatid exchange, Chinese hamster ovary (CHO) cells *in vitro*	–	–	1000	Evans & Mitchell (1981)
Sister chromatid exchange, Chinese hamster ovary (CHO) cells *in vitro*	–	–	100	Perry & Thompson (1981)
Sister chromatid exchange, Chinese hamster ovary (CHO) cells *in vitro*	+	+	500	National Toxicology Program (1996)
Sister chromatid exchange, mouse embryo cells *in vitro*	–	NT	8900	Itoh & Matsumoto (1984)
Micronucleus formation, Chinese hamster ovary (CHO) cells *in vitro*	–	NT	5000	Aardema *et al*. (2006)

Table 4.1 (continued)

Test system	Result[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Micronucleus formation, Chinese hamster lung (CHL) cells *in vitro*	–	NT	5000	Wakata *et al*. (2006)
Chromosomal aberrations, Chinese hamster ovary (CHO) cells *in vitro*	–	–	5000	National Toxicology Program (1996)
Chromosomal aberrations, mouse embryo cells *in vitro*	+	NT	8.9	Itoh & Matsumoto (1984)
Cell transformation, mouse fibroblast C3H2K cells	–	NT	100	Yoshikura & Matsushima (1981)
Cell transformation, C3H 10T1/2 mouse cells	–	–	25000	Allen *et al*. (1982)
Cell transformation, baby hamster kidney (BHK21) cells	–	+	5750	Daniel & Dehnel (1981)
Cell transformation, baby hamster kidney (BHK21) cells	NT	+	200	Styles (1981)
Unscheduled DNA synthesis, HeLa cells *in vitro*	NT	+	0.9	Martin *et al*. (1978)
Unscheduled DNA synthesis, human fibroblasts *in vitro*	+	+	0.8	Agrelo & Amos (1981)
Unscheduled DNA synthesis, HeLa cells *in vitro*	+	+	100	Martin & McDermid (1981)
Gene mutation, human lymphoblastoid TK6 cells, *HGPRT* and *TK* loci *in vitro*	–	–	12500	Hübner *et al*. (1997)
Sister chromatid exchange, human lymphocytes *in vitro*	+	–	8.9	Csukás *et al*. (1979)
Sister chromatid exchange, human lymphocytes *in vitro*	+	–	890	Csukás *et al*. (1981)
Micronucleus formation, human lymphocytes *in vitro*	–	NT	5000	Clare *et al*. (2006)
Chromosomal aberrations, human germ cells *in vitro*	–	NT	1000	Kamiguchi & Tateno (2002)
DNA strand breaks, Sprague-Dawley rat hepatocytes *in vivo*	–		500 ip	Petzold & Swenberg (1978)
DNA strand breaks, Sprague-Dawley rat brain cells *in vivo*	+		25 ip	Petzold & Swenberg (1978)
Unscheduled DNA synthesis, mouse germline cells *in vivo*	+		750 ip	Sotomayor *et al*. (1994)

Table 4.1 (continued)

Test system	Result[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Gene mutation, Chinese hamster lung cells, *in vivo*	+		1000 ip	Dean & Hodson-Walker (1979)
Gene mutation, mouse germline cells *in vivo*	–		1750 ip	Russell *et al*. (1987)
Gene mutation, mouse lung, liver and spleen cells *lacZ* operon *in vivo*	+		900 ip	Williams *et al*. (1998)
Sister chromatid exchange, mouse somatic cells *in vivo*	+		50 ip	Roberts & Allen (1980)
Sister chromatid exchange, mouse germline cells *in vivo*	+		400 ip	Roberts & Allen (1980)
Sister chromatid exchange, mouse somatic cells *in vivo*	+		193 inh and iv	Cheng *et al*. (1981a)
Sister chromatid exchange, mouse somatic cells *in vivo*	+		392 ip	Cheng *et al*. (1981b)
Sister chromatid exchange, mouse bone-marrow cells *in vivo*	+		400 ip	Allen *et al*. (1982)
Sister chromatid exchange, mouse somatic cells, alveolar macrophages and bone-marrow *in vivo*	+		300 ip	Conner & Cheng (1983)
Sister chromatid exchange, mouse bone-marrow cells *in vivo*	+		150 ip	Dragani *et al*. (1983)
Sister chromatid exchange, mouse somatic cells, lymphocytes, alveolar macrophages and bone-marrow *in vivo*	+		300 ip	Goon & Conner (1984)
Sister chromatid exchange, mouse bone-marrow cells *in vivo*	+		400 ip	Sharief *et al*. (1984)
Sister chromatid exchange, mouse lymphocytes *in vivo*	+		200 ip	Neft *et al*. (1985)
Sister chromatid exchange, mouse bone-marrow cells *in vivo*	+		300 ip	Sozzi *et al*. (1985)
Sister chromatid exchange, mouse lung cells *in vivo*	+		1000 ip	Allen *et al*. (1986)
Sister chromatid exchange, mouse fetal liver and bone-marrow cells *in vivo*	+		100 iv	Neeper-Bradley & Conner (1989, 1990)
Sister chromatid exchange, mouse somatic cells, skin and bone-marrow *in vivo*	+		0.6 ip or skin	Barale *et al*. (1992)

Table 4.1 (continued)

Test system	Result[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Sister chromatid exchange, rat bone-marrow cells *in vivo*	+		400 ip	Sharief *et al.* (1984)
Sister chromatid exchange, Chinese and Syrian golden hamster bone-marrow cells *in vivo*	+		400 ip	Sharief *et al.* (1984)
Micronucleus formation, mouse polychromatic erythrocytes *in vivo*	+		178 ip	Wild (1978)
Micronucleus formation, mouse polychromatic erythrocytes *in vivo*	+		615 ip	Salamone *et al.* (1981)
Micronucleus formation, mouse bone-marrow polychromatic erythrocytes *in vivo*	+		200 ip	Tsuchimoto & Matter (1981)
Micronucleus formation, mouse bone-marrow polychromatic erythrocytes *in vivo*	+		1000 sc	Aldovini & Ronchese (1983)
Micronucleus formation, mouse bone-marrow polychromatic erythrocytes *in vivo*	+		900 po	Ashby *et al.* (1990)
Micronucleus formation, mouse bone-marrow polychromatic erythrocytes *in vivo*	+		400 ip	Holmstrom (1990)
Micronucleus formation, mouse skin cells *in vivo*	+		2 ip	He *et al.* (1991)
Micronucleus formation, mouse bone-marrow polychromatic erythrocytes *in vivo*	+		400 po × 3	Westmoreland *et al.* (1991)
Micronucleus formation, mouse bone-marrow polychromatic erythrocytes *in vivo*	+		500 ip	Balansky *et al.* (1992)
Micronucleus formation, mouse bone-marrow polychromatic and normochromatic erythrocytes *in vivo*	+		990 ip	Sanderson & Clark (1993)
Micronucleus formation, mouse bone-marrow polychromatic erythrocytes *in vivo*	+		500 ip	Balansky (1995)

Table 4.1 (continued)

Test system	Result[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Micronucleus formation, mouse bone-marrow polychromatic erythrocytes *in vivo*	+		1000 ip	Choy *et al*. (1995, 1996)
Micronucleus formation, mouse bone-marrow polychromatic erythrocytes *in vivo*	+		25 ip	Adler *et al*. (1996)
Micronucleus formation, mouse polychromatic and normochromatic erythrocytes *in vivo*	+		200 po	National Toxicology Program (1996)
Micronucleus formation, mouse bone-marrow polychromatic erythrocytes *in vivo*	+		1000 po	Abraham *et al*. (1998)
Micronucleus formation, mouse peripheral blood normochromatic erythrocytes *in vivo*	+		400 ip	Balansky & De Flora (1998)
Micronucleus formation, mouse peripheral blood normochromatic erythrocytes *in vivo*	+		600 po, 12 wk	Director *et al*. (1998)
Micronucleus formation, mouse bone-marrow polychromatic erythrocytes *in vivo*	+		900 ip	Williams *et al*. (1998)
Micronucleus formation, mouse peripheral blood reticulocytes *in vivo*	+		500 ip	Kim *et al*. (1999)
Micronucleus formation, mouse peripheral blood polychromatic and normochromatic erythrocytes *in vivo*	+[g]		10 po 5 d/wk, 6 wk	Hoffler *et al*. (2005)
Micronucleus formation, rat polychromatic erythrocytes *in vivo*	–		2500 ip	Trzos *et al*. (1978)
Micronucleus formation, rat polychromatic erythrocytes *in vivo*	+		600 po	Westmoreland *et al*. (1991)
Micronucleus formation, rat germline spermatid cells *in vivo*	–		500 ip	Adler *et al*. (1996)
Chromosomal aberrations, mouse somatic bone-marrow, thymus and spleen cells *in vivo*	+		1000 sc	Kurita *et al*. (1969)

Table 4.1 (continued)

Test system	Result[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Chromosomal aberrations, mouse bone-marrow and spleen cells *in vivo*	+		500 sc	Miyashita *et al*. (1987)
Chromosomal aberrations, mouse somatic skin and bone-marrow cells *in vivo*	+		600 skin	Barale *et al*. (1992)
Chromosomal aberrations, mouse blood and bone-marrow somatic cells *in vivo*	–		600 po, 12 wk	Director *et al*. (1998)
Chromosomal aberrations, rat bone-marrow cells *in vivo*	+		100 ip	Topaktaş *et al*. (1996)
Dominant lethal mutation, rats	–		250 ip × 5	Jackson *et al*. (1959)
Dominant lethal mutation, mice	–		1500 ip	Bateman (1967)
Dominant lethal mutation, mice	–		1200 ip	Epstein *et al*. (1972)
Dominant lethal mutation, mice	–		100 ip	Kennedy *et al*. (1973)
Dominant lethal mutation, mice	–		2250 ip	Nomura (1982)
Dominant lethal mutation, mice	–		1750 ip	Adler *et al*. (1996)
Sperm morphology, mice	–		1000 ip × 5	Wyrobek & Bruce (1975)
Sperm morphology, mice	–		1000 ip × 5	Topham (1981)

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

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro formations, µg/mL; in-vivo formations, mg/kg bw/day; d, day; inh, inhalation; ip, intraperitoneal; iv, intravenous; po, oral; sc, subcutaneous; wk, week

[c] Two of 7 formations weakly positive without exogenous metabolic system (S9) and 1 of 7 formations weakly positive with S9

[d] Four of 7 formations negative without exogenous metablic system (S9) and two of 7 formations weakly positive with S9

[e] Positive in some, but not all formations performed at different laboratories

[f] One of 5 formations strongly positive

[g] Effect largely absent in*CYP2E1*-null mice

Some reports have indicated that ethyl carbamate can cause DNA damage in mammalian cells *in vitro* and *in vivo*. Ethyl carbamate and/or its metabolites can bind to nucleic acids *in vivo*. Boyland and Williams (1969) showed that, after intraperitoneal injection of radiolabelled ethyl or carboxyethyl carbamate to mice, liver and lung RNA were labelled. It was also noted that the ability of ethyl carbamate to bind to nucleic acids correlates with its organ-, sex- and strain-specific carcinogenic potency (Fossa *et al.*, 1985). Sotomayor *et al.* (1994) administered 10–1000 mg/kg bw [^{3}H]ethyl carbamate to male mice intraperitoneally and measured DNA binding and unscheduled DNA synthesis in the liver and testis 12 hours later. A linear increase in the binding of labelled ethyl carbamate to DNA was detected in both organs, although the binding increased more rapidly in the liver at lower doses. Unscheduled DNA synthesis was elevated in early spermatids only with the 750-mg/kg bw dose.

Ribovich *et al.* (1982) demonstrated that 1,N^6-ethenoadenosine and 3,N^4-ethenocytidine were formed in the RNA of liver after intraperitoneal administration of radiolabelled [ethyl-1,2-^{3}H]ethyl carbamate to mice. Following single and multiple intraperitoneal injections of ethyl carbamate or its metabolites, vinyl carbamate or vinyl carbamate oxide, the formation of 1,N^6-ethenodeoxyadenosine and 3,N^4-ethenodeoxycytidine was increased in the liver and lung DNA of several mouse strains (Fernando *et al.*, 1996). Vinyl carbamate was about threefold more potent in inducing etheno-DNA adducts in either the liver or lung. Recently, Beland *et al.* (2005) reported that the levels of 1,N^6-ethenodeoxyadenosine in hepatic DNA were increased by exposure to ethyl carbamate (90 ppm [90 μg/mL], 4 weeks in the drinking-water) but were lower when 5% ethanol served as the vehicle. In the same study, neither ethyl carbamate nor ethanol affected the levels of 1,N^6-ethenodeoxyadenosine or 3,N^4-ethenodeoxycytidine in lung DNA.

It was also suggested that *N*-7-(2-oxoethyl)guanine may be a key DNA adduct formed after exposure to ethyl carbamate (Scherer *et al.*, 1986). These authors also showed that vinyl carbamate is a much more potent inducer of this adduct than ethyl carbamate. Svensson (1988) reported the formation of 2-oxoethyl haemoglobin and the DNA adduct *N*-7-(2-oxoethyl)guanine in mice treated with ethyl carbamate and the number of protein adducts increased linearly with dose. The *N*-7-(2-oxoethyl)guanine adduct is not considered to be pro-mutagenic but it was suggested that it may lead to cross-linking in DNA (Conner & Cheng, 1983), a mechanism that may be involved in the sister chromatid exchange induced by ethyl carbamate in multiple test systems.

4.5 Mechanistic considerations

The following are potential mechanisms that are not mutually exclusive.

4.5.1 *Genotoxicity*

The carcinogenicity of ethyl carbamate is thought to be mediated via a bioactivation pathway in which it is oxidized sequentially by CYP2E1 to vinyl carbamate and vinyl carbamate epoxide (Dahl *et al.*, 1978). Vinyl carbamate epoxide is a DNA-reactive species that can yield promutagenic etheno-DNA adducts. In support of this hypothesis, vinyl carbamate has been shown to induce more hepatocellular carcinomas than ethyl carbamate (Dahl *et al.*, 1980) and vinyl carbamate epoxide is more hepatocarcinogenic than vinyl carbamate (Park *et al.*, 1993). DNA adducts indicative of exposure to vinyl carbamate epoxide have been detected in the liver DNA of mice treated with ethyl carbamate (Beland *et al.*, 2005), vinyl carbamate and vinyl carbamate epoxide (Fernando *et al.*, 1996). In addition, hepatocellular adenomas and carcinomas induced in B6C3F$_1$ mice by ethyl and vinyl carbamate have a characteristic increase in CAA to CTA mutations at codon 61 of the H-*Ras* oncogene compared with CAA to AAA mutations that are typically found in spontaneous tumours (Wiseman *et al.*, 1986; Dragani *et al.*, 1991). Such mutations are consistent with the formation of 1,N^6-ethenodeoxyadenosine, which has been shown to lead to A→T transversion mutations (Levine *et al.*, 2000). In addition, it has been suggested that a potential DNA-cross-linking alkylating adduct, *N*-7-(2-oxoethyl)guanine, may be formed after exposure to ethyl carbamate *in vivo* (Scherer *et al.*, 1986).

4.5.2 *Cell proliferation*

Treatment with ethyl carbamate has been shown to induce cell proliferation in mouse lung (Yano *et al.*, 1997, 2000) and liver (Beland *et al.*, 2005). Cell proliferation can occur either as a regenerative response to cytotoxicity or via the induction of other molecular pathways. In the mouse lung, ethyl carbamate has been purported to act via the induction of ornithine decarboxylase and subsequent polyamine accumulation (Yano *et al.*, 1997), which are events that are thought to be involved in stimulation of the cell cycle. The proliferative effects of ethyl carbamate in the liver seem to be sex-specific, since hepatocellular proliferation was observed only in female mice (Beland *et al.*, 2005). The fact that female mice in the same study had a greater relative increase in the incidence of hepatocellular tumours following administration of ethyl carbamate may suggest that formation of the genotoxic metabolites of ethyl carbamate (see above), coupled with a greater rate of cell replication, contributes to the tumour response. It was shown that ethyl carbamate-induced increases in cell proliferation in the liver and lung are dependent on CYP2E1 because no effect was observed in *CYP2E1*-null mice (Hoffler *et al.*, 2005).

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