3. Studies of Cancer in Experimental Animals

Previous evaluation

Ethyl carbamate was evaluated by an IARC Working Group in February 1974 (IARC, 1974). It was also the subject of a very extensive review (Salmon & Zeise, 1991). Both reviews evaluated bioassays in which mice, rats and hamsters were exposed to ethyl carbamate by oral, dermal, subcutaneous and/or intraperitoneal routes.

Mice treated orally with ethyl carbamate had an increased incidence of lung adenomas, carcinomas and squamous-cell tumours, lymphomas (mainly lymphosarcomas), mammary gland adenocarcinomas and carcinomas, leukaemia and Harderian gland adenomas and angiomas. When oral administration was accompanied by topical application of the tumour promoter 12-*O*-tetradecanoylphorbol-13 acetate (TPA), the incidence of skin papillomas and squamous-cell carcinomas was significantly increased. Rats treated orally with ethyl carbamate had an increased incidence of Zymbal gland and mammary gland carcinomas. Hamsters treated orally with ethyl carbamate showed an increased incidence of skin melanotic tumours, forestomach papillomas, mammary gland adenocarcinomas, liver hepatomas, liver and spleen haemangiomas and carcinomas of the thyroid, ovary and vagina.

Topical application of ethyl carbamate to mice resulted in a significant increase in the incidence of lung adenomas and mammary gland carcinomas.

Subcutaneous administration of ethyl carbamate induced a significant increase in the incidence of lung adenomas in adult mice and hepatomas in newborn mice. When the treatment was followed by topical application of croton oil, a significant increase in the incidence of skin papillomas was observed.

Intraperitoneal administration of ethyl carbamate to adult mice resulted in a significant increase in the incidence of lung adenomas, hepatomas and skin papillomas. Similar treatment in newborn mice induced lymphomas, lung adenomas, hepatomas, Harderian gland tumours and stromal and epithelial tumours of the ovary.

Mice exposed transplacentally to ethyl carbamate developed an increased incidence of lung tumours, hepatomas and ovarian tumours.

Subsequent bioassays are summarized below.

3.1 Oral administration

3.1.1 *Mouse*

Groups of 50 male B6C3F$_1$ mice, 6 weeks of age, were given 0, 0.6, 3, 6, 60 or 600 ppm ethyl carbamate (> 99% pure) in the drinking-water for 70 weeks. Mice that survived more than 23 weeks were included in the analysis of tumours (i.e. effective number of mice). The effective number of mice was 49, 49, 48, 50, 50 and 44 for the 0-, 0.6-, 3-, 6-, 60- and 600-ppm ethyl carbamate dose groups, respectively. The mean survival of the 600-ppm dose group was significantly shorter than that of the control group (39.2 weeks versus 69.5 weeks, respectively; $P < 0.01$, Student's *t*-test). The other groups had mean survival times of ≥ 65.5 weeks. All mice were autopsied and histological examinations were conducted. Ethyl carbamate caused dose-related increases in the incidence of lung alveolar/bronchiolar adenomas and carcinomas, liver haemangiomas and angioasarcomas and heart haemangiomas. The incidence of lung alveolar/bronchiolar adenoma was 9/49 (18%), 4/49 (8%), 7/48 (15%), 8/50 (16%), 34/50 (68%) and 42/44 (95%) for the 0-, 0.6-, 3-, 6-, 60- and 600-ppm ethyl carbamate-treated groups, respectively; the increase at 60 and 600 ppm ethyl carbamate was significant ($P < 0.01$) compared with the control group. Lung alveolar/bronchiolar carcinoma was only observed in the 600-ppm ethyl carbamate-treated group (6/44; 14%), an incidence that was significant. Liver haemangioma occurred in the 60- and 600-ppm ethyl carbamate-treated groups (2/50 (4%) and 20/44 (45%), respectively), and the increase in the 600-ppm group was significant ($P < 0.01$). Liver angiosarcoma developed in the 6-, 60- and 600-ppm ethyl carbamate-treated groups at incidences of 2/50 (4%), 2/50 (4%) and 11/44 (25%), respectively; the latter was a significant increase compared with the control group ($P < 0.01$). Heart haemangioma occurred only in the mice treated with 600 ppm ethyl carbamate (4/44; 9%), an incidence that was significant ($P < 0.05$) (Inai *et al*., 1991).

Groups of 48 male and 48 female B6C3F$_1$ mice, 4 weeks of age, were given 0, 10, 30 or 90 ppm ethyl carbamate (> 99% pure) in the drinking-water for 104 weeks. The administration of ethyl carbamate caused a dose-dependent decrease in survival in both male and female mice, and the effect was significant at 30 and 90 ppm ethyl carbamate. Complete necropsies were performed on all mice and histological examinations were conducted. The incidence of tumours in males treated with 0-, 10-, 30- and 90-ppm, respectively, was: lung alveolar/bronchiolar adenomas or carcinomas, 5/48 (10%), 18/48 (37%), 29/47 (62%) and 37/48 (77%) (the increases at 10, 30 and 90 ppm ethyl carbamate were significant; $P < 0.05$); hepatocellular adenomas or carcinomas, 12/46 (26%), 18/47 (38%), 24/46 (52%) and 23/44 (52%) (the increases at 30 and

90 ppm ethyl carbamate were significant; $P < 0.05$); liver haemangiosarcomas, 1/46 (2%), 2/47 (4%), 5/46 (11%) and 13/44 (29%) (the increase at 90 ppm ethyl carbamate was significant; $P < 0.05$); Harderian gland adenomas or carcinomas, 3/47 (6%), 12/47 (25%), 30/47 (64%) and 38/47 (81%) (the increases at all three doses were significant; $P < 0.05$); skin squamous-cell papillomas or carcinomas, 0/47, 1/48 (2%), 3/47 (6%) and 6/48 (12%) (the increase at 90 ppm ethyl carbamate was significant; ($P < 0.05$); forestomach squamous-cell papillomas, 0/46, 2/47 (14%), 3/44 (7%) and 5/45 (11%) (the increase at 90 ppm ethyl carbamate was significant; $P < 0.05$); and heart haemangiosarcomas, 0/48, 0/48, 1/47 (2%) and 5/48 (10%) (the increase at 90 ppm ethyl carbamate was significant; $P < 0.05$). The incidence of tumours in female mice treated with 0-, 10-, 30- and 90-ppm, respectively, was: lung alveolar/bronchiolar adenomas or carcinomas, 6/48 (12%), 8/48 (17%), 28/48 (53%) and 39/47 (83%) (increases at 30 and 90 ppm ethyl carbamate were significant; $P < 0.05$); hepatocellular adenomas or carcinomas, 5/48 (10%), 11/47 (23%), 20/47 (43%) and 19/47 (40%) (the increases at 30 and 90 ppm ethyl carbamate were significant; $P < 0.05$); liver haemangiosarcoma, 0/48, 0/47, 1/47 (2%) and 7/47 (15%) (the increase at 90 ppm ethyl carbamate was significant; $P < 0.05$); mammary gland adenocarcinomas, 4/47 (8%), 3/46 (6%), 3/46 (6%) and 11/48 (23%) (the increase at 90 ppm ethyl carbamate was significant; $P < 0.05$); mammary gland adenoacanthomas, 0/47, 1/46 (2%), 1/46 (2%) and 11/48 (23%) (the increase at 90 ppm ethyl carbamate was significant; $P < 0.05$); Harderian gland adenomas or carcinomas, 3/48 (6%), 11/48 (23%), 19/48 (40%) and 30/48 (62%) (the increases at all three doses were significant; $P < 0.05$); and ovary granulosa-cell tumours, 0/48, 0/46, 2/46 (4%) and 5/39 (13%) (the increase at 90 ppm ethyl carbamate was significant; $P < 0.05$) (National Toxicology Program, 2004; Beland *et al*., 2005).

A study was conducted to compare the carcinogenicity of ethyl carbamate in mice that are proficient and deficient in cytochrome-P450 (CYP) 2E1. Groups of 28–30 male *Cyp2e1*+/+ and *Cyp2e1*–/– mice, 5–6 weeks of age, were administered by gavage 0, 1, 10 or 100 mg/kg body weight (bw) ethyl carbamate (purity, > 98%) once a day on 5 days per week for 6 weeks. The ethyl carbamate was dissolved in water and administered in a volume of 10 mL/kg bw. Twenty-four hours after the last treatment, 14–15 mice per group were killed. The remaining 14–15 mice per group were held for 7 months. Complete gross necropsy and microscopic examination were performed on all mice. Seven months after the end of treatment, liver tumours (haemangiomas and haemangiosarcomas) were observed in male *Cyp2e1*+/+ mice treated with 100 mg/kg bw ethyl carbamate (5/15 (33%) and 8/15 (53%) compared with 0/14 and 0/14, respectively, in control male *Cyp2e1*+/+ mice). The increased incidence was significant ($P < 0.05$ and < 0.01, respectively). Liver haemangioma was detected in a single *Cyp2e1*–/– mouse (1/15; 7%) treated with 100 mg/kg bw ethyl carbamate. The difference in the incidence of liver haemangiosarcomas was significant when *Cyp2e1*+/+ mice were compared with *Cyp2e1*–/– mice treated with 100 mg/kg bw ethyl carbamate (8/15 (53%) versus 0/15; $P = 0.0011$); the difference in the incidence of liver haemangioma was marginally significant (5/15 (33%) versus 1/15 (7%); $P = 0.0843$). In male *Cyp2e1*+/+ mice,

the incidence of bronchioalveolar adenoma was 0/14, 3/14 (21%), 14/14 (100%) and 14/15 (93%) in the control, low-dose, mid-dose and high-dose groups, and tumour multiplicities were 0, 1.0, 2.5 and 15.4 tumours/lung, respectively. The incidence of bronchioalveolar adenoma was significantly increased with doses of 10 and 100 mg/kg bw ethyl carbamate ($P < 0.01$) and there was a significant variation in the tumour multiplicity across doses ($P < 0.0001$). In the respective groups of male *Cyp2e1–/–* mice, the incidence of bronchioalveolar adenoma was 0/15, 0/15, 4/14 (29%) and 9/15 (60%), and tumour multiplicities were 0, 0, 1.0 and 2.4 tumours/lung. The incidence of bronchioalveolar adenoma was significantly increased with doses of 10 and 100 mg/kg bw ethyl carbamate ($P < 0.05$ and < 0.01; respectively). The difference in the incidence of bronchioalveolar adenoma was significant when *Cyp2e1+/+* mice were compared with *Cyp2e1–/–* mice treated with 10 and 100 mg/kg bw ethyl carbamate ($P = 0.0001$ and 0.04, respectively). The difference in the multiplicity of bronchioalveolar adenoma was also significant when *Cyp2e1+/+* mice were compared with *Cyp2e1–/–* mice treated with 10 and 100 mg/kg bw ethyl carbamate ($P = 0.0145$ and < 0.0001, respectively). A single case of bronchioalveolar carcinoma was detected in a *Cyp2e1+/+* mouse treated with 100 mg/kg bw ethyl carbamate. In male *Cyp2e1+/+* mice, the incidence of Harderian gland adenoma was 1/14 (7%), 4/14 (29%), 14/14 (100%) and 13/15 (87%) in control, low-dose, mid-dose and high-dose groups, respectively, and was significantly increased at 10 and 100 mg/kg bw ethyl carbamate ($P < 0.01$). That in male *Cyp2e1–/–* mice was 0/15, 1/15 (7%), 2/14 (14%) and 12/15 (80%), respectively and was significantly increased with the dose of 100 mg/kg bw ethyl carbamate ($P < 0.01$). The difference in the incidence of Harderian gland adenoma was significant when *Cyp2e1+/+* mice were compared with *Cyp2e1–/–* mice treated with 10 mg/kg bw ethyl carbamate ($P < 0.0001$) (Ghanayem, 2007).

3.1.2 *Monkey*

A group of neonatal cynomologus, rhesus and/or African green monkeys [sex, number and distribution not specified] was administered 250 mg/kg bw ethyl carbamate [purity not specified] orally in sterile water [volume not specified] on 5 days per week for 5 years. Thirty-two monkeys survived the first 6 months of treatment, at which time they typically were weaned. Some of the monkeys also received 7–10 weekly courses of whole-body radiation (50 rad per course). None of the monkeys survived after 5 years of treatment. Complete necropsies were performed on all animals. Six of the 32 (19%) monkeys developed one or more primary tumours. The tumours included adenocarcinoma of the lung, pancreas, bile ducts and small intestine, hepatocellular adenoma and carcinoma, haemangiosarcoma of the liver, ependymoma, pheochromocytoma, endocervical adenofibroma and squamous papilloma of the pouch. The specific incidences were not reported. Only two of the six (33%) monkeys that had malignant tumours had been irradiated. A concurrent control group did not appear to be included. Autopsy records were available for 373 breeders and ‘normal controls’.

Nineteen of these monkeys developed malignant and/or benign tumours. While some tumours occurred in both untreated and ethyl carbamate-treated monkeys (e.g. adenocarcinoma of the pancreas and intestine), hepatocellular adenoma and carcinoma and adenocarcinoma of the lung were only found in ethyl carbamate-treated monkeys (Thorgeirsson *et al*., 1994). [The Working Group noted the poor design and reporting of the study.]

3.2 Skin application

Mouse

A study was conducted to determine whether or not ethyl carbamate would act as an enhancer of skin carcinogenesis induced by 7,12-dimethylbenz[*a*]anthracene (DMBA). A group of 16 male and 16 female hairless *hr/hr* Oslo mice [age not specified] was treated topically once with 51.2 μg DMBA [purity not specified] in 100 μL acetone and were observed for 60 weeks. An additional group of the same number of mice was treated identically with DMBA and then, after a 2-week period, were treated topically twice a week for 50 weeks with 100 μL of a solution of 10% ethyl carbamate [purity not specified] in acetone. An additional group of the same number of mice was not treated with DMBA, but was treated with ethyl carbamate for a period of 60 weeks. Gross necropsies and histology were performed. Tumour rates (the percentage of tumour-bearing mice in relation to the number of mice alive at the appearance of the first tumour related to time) and yields (the cumulative occurrence of all skin tumours related to time) were analysed statistically. Mice treated with DMBA alone had a total of 21 skin tumours (primarily papillomas, but also carcinomas and atypical keratoacanthomas) in 11 mice and no lung adenomas; mice treated with ethyl carbamate alone had a total of eight skin tumours in five mice and 79 lung adenomas in 22 mice; and mice treated with DMBA and ethyl carbamate had a total of 60 skin tumours in 16 mice and 121 lung adenomas in 23 mice. Treatment with DMBA and ethyl carbamate induced a significantly higher number of skin tumours than treatment with DMBA alone (Iversen, 1991).

3.3 Inhalation exposure

Mouse

Groups of female JCL:ICR mice [number per group not specified], 28 days of age, were exposed to air containing 0.25 μg/mL ethyl carbamate [purity not specified] for 1, 3, 5 or 10 days or air containing 1.29 μg/mL ethyl carbamate for 0.25, 1, 2, 4 or 5 days. Groups of male JCL:ICR mice, 28 days of age, were exposed to air containing 0.25 μg/mL ethyl carbamate for 10 days (50 mice) or air containing 1.29 μg/mL

ethyl carbamate for 4 days (47 mice). Concurrent controls were exposed to air only. Female mice were killed 5 months after the exposure period and male mice were killed 12 months after the exposure period. Histological analyses were performed. Female mice exposed by inhalation to 0.25 μg/mL ethyl carbamate had a lung tumour incidence [tumour type not specified] and tumour multiplicity (tumours per lung) of 27/51 (53%) and 1.08 ± 0.39 (mean ± 95% confidence interval [CI]) after exposure for 1 day, 44/51 (86%) and 5.29 ± 1.28 after exposure for 3 days, 46/53 (87%) and 7.56 ± 2.05 after exposure for 5 days and 9/11 (82%) and 17.8 ± 4.6 after exposure for 10 days. In each of the exposed groups, the lung tumour incidence [$P < 0.0001$; one-tailed Fisher's exact test] and tumour multiplicity ($P < 0.05$) were significantly increased compared with the concurrent control group, which had values of 2/51 (4%) and 0.04, respectively. Female mice exposed by inhalation to 1.29 μg/mL ethyl carbamate had a lung tumour incidence [tumour type not specified] and tumour multiplicity of 38/79 (48%) and 0.67 ± 0.20 after exposure for 0.25 days, 37/40 (92%) and 10.7 ± 2.9 after exposure for 1 day, 66/70 (94%) and 18.6 ± 3.8 after exposure for 2 days, 81/86 (94%) and 10.6 ± 2.6 after exposure for 4 days and 18/18 (100%) and 12.2 ± 3.9 after exposure for 5 days. In each of the exposed groups, the lung tumour incidence [$P < 0.0001$; one-tailed Fisher's exact test] and tumour multiplicity ($P < 0.05$) were significantly increased compared with the concurrent control group, which had values of 2/51 (4%) and 0.04, respectively. Male mice exposed by inhalation to 0.25 μg/mL ethyl carbamate for 10 days had a lung adenocarcinoma incidence of 40/50 (80%), of which 11 (22%) showed signs of invasion or metastasis. Male mice exposed by inhalation to 1.29 μg/mL ethyl carbamate for 4 days had a lung adenocarcinoma incidence of 14/40 (35%). This group was composed of 47 mice, of which seven died within 7 days of being treated. In each of the exposed groups, the lung adenocarcinoma incidence was significantly increased ($P < 0.01$) compared with the control group, which had an incidence of 1/51 (2%). [The Working Group questioned the high incidence of adenocarcinomas associated with high survival.] The incidence of leukaemia in female mice exposed by inhalation to 0.25 μg/mL ethyl carbamate was 3/51 (6%) after exposure for 1 day, 2/51 (4%) after exposure for 3 days, 5/53 (9%) after exposure for 5 days and 0/11 after exposure for 10 days. The incidence of leukaemia in mice exposed for 5 days was significantly greater [$P = 0.0312$; one-tailed Fisher's exact test] than that in concurrent controls, which had an incidence of 0/51. Female mice exposed by inhalation to 1.29 μg/mL ethyl carbamate had an incidence of leukaemia of 2/79 (2%) after exposure for 0.25 days, 1/40 (2%) after exposure for 1 day, 12/70 (17%) after exposure for 2 days, 18/86 (21%) after exposure for 4 days and 3/18 (17%) after exposure for 5 days. The incidence in mice in each of the groups exposed for 2 or more days was significantly greater [$P \leq 0.0156$; one-tailed Fisher's exact test] than that in the concurrent control group, which had an incidence of 0/51. The incidence of leukaemia in male mice exposed by inhalation to 0.25 μg/mL ethyl carbamate for 10 days was 5/50 (10%). Male mice exposed by inhalation to 1.29 μg/mL ethyl carbamate for 4 days had an incidence of 8/40 (20%). In each of the exposed groups, the incidence of leukaemia was significantly increased [$P \leq 0.0264$;

one-tailed Fisher's exact test] compared with the control group, which had an incidence of 0/51. The incidence of uterine haemangioma in female mice exposed by inhalation to 1.29 μg/mL ethyl carbamate was 0/79 after exposure for 0.25 days, 1/40 (2%) after exposure for 1 day, 2/70 (3%) after exposure for 2 days, 8/86 (9%) after exposure for 4 days and 0/18 after exposure for 5 days. The incidence of uterine haemangioma in mice exposed for 4 days was significantly greater [$P = 0.0212$; one-tailed Fisher's exact test] than that in the concurrent control group, which had an incidence of 0/51. A single uterine haemangioma 1/51 (2%) was also observed in female mice exposed to 0.25 μg/mL ethyl carbamate for 3 days. The incidence of hepatoma in male mice exposed by inhalation to 0.25 μg/mL ethyl carbamate for 10 days was 6/50 (12%). In male mice exposed by inhalation to 1.29 μg/mL ethyl carbamate for 4 days, the incidence of hepatoma was 3/40 (7%). The incidence of hepatoma in the mice exposed to 0.25 μg/mL ethyl carbamate was marginally increased [$P = 0.0529$; one-tailed Fisher's exact test] compared with the control group, which had an incidence of 1/51 (2%) (Nomura *et al*., 1990).

3.4 Other exposures

3.4.1 *Pre-conception*

Mouse

A study was conducted to investigate whether pre-conception exposure of sperm cells to ethyl carbamate resulted in an increased risk for cancer in either untreated progeny or progeny treated with ethyl carbamate. Groups of 45 male CBA/JNCrj mice, 9 weeks of age, received two subcutaneous injections of 10 μL/g bw saline or 10 μL/g bw saline that contained 500 μg/kg bw ethyl carbamate (purity, > 99%) at a 24-hour interval. At 1, 3 and 9 weeks after treatment (i.e. at different stages of spermatogenesis), each male mouse was mated for 4 days with three untreated virgin 12-week-old female CBA/JNCrj mice. When the progeny were 6 weeks of age, one half was treated once with a subcutaneous injection of 10 μL/g bw saline and the other half was treated with 10 μL/g bw saline that contained 100 μg/kg bw ethyl carbamate. The mice were then kept for lifetime. The mean lifetime for the male mice, including the parental males, was 80–91 weeks, and that for the female mice, including the parental females, was 87–94 weeks. Statistical analyses indicated only sporadic differences in survival when ethyl carbamate-treated groups were compared with their appropriate control groups. Complete necropsies and histological examinations were conducted on all animals. Paternal treatment with ethyl carbamate caused a significant increase (98%) in the incidence of lung tumours (bronchioloalveolar adenomas and adenocarcinomas) in parental male mice compared with 22% in the 45 controls. Male F_1 mice treated with saline had a lung tumour incidence of 17–24% (71–135 mice per group); those treated with ethyl carbamate had a lung tumour incidence of 43–60% (83–124 mice per group). Paternal treatment had no consistent effect on lung-tumour incidence in

male F_1 mice. Male F_1 mice treated with ethyl carbamate had a significantly increased incidence of lung tumours [$P \leq 0.0004$; one-tailed Fisher's exact test], irrespective of the paternal treatment. Female F_1 mice treated with saline had a lung tumour incidence of 11–24% (59–111 mice per group) compared with 32–43% (81–104 mice per group) in those treated with ethyl carbamate. Paternal treatment with ethyl carbamate had no effect on the incidence of lung tumours in female F_1 mice. Female F_1 mice treated with ethyl carbamate had a significantly increased lung-tumour incidence [$P \leq 0.0168$; one-tailed Fisher's exact test], irrespective of the paternal treatment, with the exception of mice resulting from the 3-week mating of ethyl carbamate-treated F_0 male mice, which may be a spurious result. Paternal treatment with ethyl carbamate caused a significant increase (76%) in the incidence of liver tumours (hepatocellular adenomas and adenocarcinomas) in the parental male mice, compared with 53% in the 45 controls. Male F_1 mice treated with saline had a liver-tumour incidence of 54–66% compared with those treated with ethyl carbamate (56–70%). Paternal treatment with ethyl carbamate had no effect on the liver-tumour incidence in male F_1 mice. The incidence of liver tumours in male F_1 mice treated with ethyl carbamate did not differ from that in mice treated with saline, irrespective of the paternal treatment. Female F_1 mice treated with saline had a liver-tumour incidence of 2–7%; those treated with ethyl carbamate had a lung tumour incidence of 2–12%. Paternal treatment with ethyl carbamate had no consistent effect on lung-tumour incidence in female F_1 mice. Treatment of female F_1 mice with ethyl carbamate had no consistent effect on the incidence of liver tumours. Lymphomas and histocytic sarcomas occurred in both F_0 male mice (7%) and their F_1 offspring (5–14% in males; 11–20% in females). The haematopoietic tumour incidence was not affected by treatment with ethyl carbamate in either the F_0 male mice or their F_1 offspring of either sex (Mohr *et al*., 1999).

Male Swiss Cr:NIH(S) mice, 6 weeks of age [number not specified], received a single intraperitoneal injection of distilled water [volume not specified] or distilled water that contained 1.5 g/kg bw ethyl carbamate [purity not specified]. Two weeks later, each male mouse was housed with five 8-week-old female mice for an unspecified period of time. This timing was selected to ensure that the sperm used in fertilization would have been exposed postmeiotically, a stage of high sensitivity to pre-conception carcinogenic effects. Three weeks later, female mice that were visibly pregnant were housed individually and allowed to give birth. The offspring were weaned at 4 weeks. The experiment lasted until the last animal died, which was approximately 157 weeks after birth. Seventy-one per cent of the female mice placed with control male mice became pregnant. For the carcinogenesis study, 71 female offspring, arising from 23 litters, and 48 male offspring, arising from 14 litters, were used. These litters were the product of 11 sires. Sixty-six percent of the female mice placed with ethyl carbamate-treated male mice became pregnant. For the carcinogenesis study, 78 female offspring, arising from 20 litters, and 54 male offspring, arising from 20 litters, were used. These litters were the product of 12 sires. Paternal treatment with ethyl carbamate resulted in the induction of adrenal gland tumours in both the male and female offspring. The

incidence was 6/132 (5%), of which five were pheochromocytomas and one was a cortical adenoma. These tumours were not detected in the offspring (0/119) of control male mice that had been treated with distilled water. The increase in the incidence of both pheochromocytomas ($P = 0.039$) and total adrenal gland tumours [$P = 0.020$; one-tailed Fisher's exact test] was significant. Treatment with ethyl carbamate resulted in the induction of glandular stomach tumours in the male offspring. In the 54 male experimental mice, 10 (18%) glandular stomach lesions developed, of which three (6%) were adenomas, three were carcinomas and four (7%) were atypical hyperplasias. In the 48 male control mice, two (4%) adenomas developed. The increase in the incidence of combined neoplastic and non-neoplastic lesions was significant ($P = 0.024$) (Yu *et al*., 1999).

3.4.2 *Transplacental exposure*

Mouse

A group of 25 pregnant Swiss Webster mice, 10 weeks of age, received a single intravenous injection of 3.3 mmol/kg bw ethyl carbamate [purity not specified] in 250 μL phosphate-buffered saline on gestational day 14. A control group of 22 pregnant female mice of the same age received two injections (250 and 100 μL) of the phosphate-buffered saline only. An additional group of 30 virgin female mice was treated with 3.3 mmol/kg bw ethyl carbamate in phosphate-buffered saline and a further group of 29 virgin female mice was injected with phosphate-buffered saline alone. All injections were followed by a 'chaser' injection of 100 μL phosphate-buffered saline. Six months after the pregnant mice gave birth, the dams, their offspring and the virgin female mice were killed to determine lung-tumour incidence by gross analysis of the lungs. One control dam died before the scheduled killing. Survival in the offspring was not indicated. The incidence of lung adenomas in 21 control dams was 28.6%, with a tumour multiplicity of 0.33 tumours per mouse. The comparable values in the 96 male and 72 female offspring were 10.4% and 0.12 tumour per mouse and 16.6% and 0.19 tumour per mouse, respectively. The incidence of lung adenomas in 20 dams treated with ethyl carbamate was 95.0%, with a tumour multiplicity of 10.5 tumours per mouse. The comparable values in the 90 male and 70 female offspring were 45.0% and 0.96 tumour per mouse and 57.1% and 1.3 tumours per mouse, respectively. The incidence of lung adenomas in 29 control virgin females was 44.8%, with a tumour multiplicity of 0.75 tumour per mouse. The comparable values for 30 virgin females treated with ethyl carbamate were 100% and 6.2 tumours per mouse (Neeper-Bradley & Conner, 1992).

3.5 Metabolites of ethyl carbamate

Previous evaluation

During the review of ethyl carbamate by a previous IARC Working Group (IARC, 1974), the carcinogenicity of ethyl carbamate metabolites was considered briefly. The Working Group concluded that ethyl carbamate needed metabolism to exert its carcinogenicity. Bioassays have been conducted on several oxidized metabolites of ethyl carbamate, and these are summarized below.

3.5.1 *Oral administration*

Mouse

Groups of 20 or 25 male and 20 or 25 female Swiss mice, 2–3 months of age, were given a single oral dose of 25 mg ethyl carbamate [purity not specified] or 25 mg *N*-hydroxyethyl carbamate [purity not specified] in distilled water [volume not specified]. A control group of 46 mice remained untreated. Four days after the initial treatment, all groups received twice-weekly dermal applications of 5% croton oil in liquid paraffin [volume not specified]. The incidence and multiplicity of skin tumours were assessed after 20 and 40 weeks of croton-oil application; those of lung tumours were assessed after 40 weeks of croton-oil application. Histopathology was conducted on the lungs. Survival was ≥ 90% after 20 weeks and ≥ 80% after 40 weeks of croton oil application. After 20 weeks, the incidence and multiplicity (± standard deviation [SD]) of skin tumours were 16/18 (89%) and 1.5 ± 0.2 for mice treated with 25 mg ethyl carbamate and 12/25 (48%) and 0.7 ± 0.2 for mice treated with 25 mg *N*-hydroxyethyl carbamate versus 3/45 (7%) and 0.07 ± 0.05 for mice treated with croton oil only. The skin tumour incidence [$P \leq 0.0001$; one-tailed Fisher's exact test] and tumour multiplicity [$P < 0.001$; one-way ANOVA followed by SNK test] in each of the treatment groups were significantly increased compared with the croton oil control mice. The skin tumour incidence [$P = 0.0088$; two-tailed Fisher's exact test] and tumour multiplicity [$P < 0.001$; one-way ANOVA followed by SNK test] in mice treated with 25 mg ethyl carbamate were significantly greater than those in mice treated with the approximately equimolar amount of 25 mg *N*-hydroxyethyl carbamate. After 40 weeks of croton oil application, the incidence and multiplicity (± SD) of skin tumours were 16/18 (89%) and 1.6 ± 0.3 for mice treated with 25 mg ethyl carbamate and 19/20 (95%) and 1.5 ± 0.3 for mice treated with 25 mg *N*-hydroxyethyl carbamate versus 11/44 and 0.4 ± 0.1 for mice treated with croton oil only. The skin-tumour incidence [$P < 0.0001$; one-tailed Fisher's exact test] and tumour multiplicity [$P < 0.001$; one-way ANOVA followed by SNK test] in each of the treatment groups were significantly increased compared with the croton-oil control mice. After 40 weeks of croton-oil application, the incidence and multiplicity (± standard deviation) of lung tumours were 12/18 (67%) and 3.4 ± 1.3 for mice treated with 25 mg ethyl carbamate and 9/20 (45%) and 0.75 ± 0.3 for mice

treated with 25 mg *N*-hydroxyethyl carbamate versus 2/42 (5%) and 0.05 ± 0.03 for mice treated with croton oil only. The lung-tumour incidence [$P \leq 0.0003$; one-tailed Fisher's exact test] and tumour multiplicity [$P < 0.001$; one-way ANOVA followed by SNK test] in each of the treatment groups were significantly increased compared with the croton-oil control mice. The tumour multiplicity in mice treated with 25 mg ethyl carbamate was significantly greater than that in mice treated with the approximately equimolar amount of 25 mg *N*-hydroxyethyl carbamate [$P < 0.001$; two-tailed Fisher's exact test] (Berenblum *et al*., 1959).

3.5.2 *Dermal application*

Mouse

Groups of 40 female CD-1 mice, 6–8 weeks of age, were pretreated topically on the shaved back with 1.2 mg croton oil in 200 μL redistilled acetone. Eighteen to 24 hours later, each mouse was treated topically with 5 or 60 mg ethyl carbamate (> 99% pure by gas chromatography) or 5 mg vinyl carbamate (melting-point, 53–54°C; purity verified by elemental analysis, MS, infrared and nuclear magnetic resonance spectroscopy) in 200 μL acetone or the solvent alone. The application of the carbamate compounds or solvent was repeated 1 week later. One week after the second application, all mice were treated twice weekly with 900 μg croton oil in 150 μL acetone. The negative controls received the croton oil pre- and post-treatment, but were given the vehicle only with no carbamate. The experiment lasted 32 weeks, at which time ≥ 88% of the mice were still alive. All animals were subjected to gross necropsy. The lungs were fixed in formalin, and adenomas on the surface (≥ 1 mm in diameter) were counted. Representative tumours were fixed, sectioned and stained with haematoxylin and eosin. The incidence of skin papillomas and the average number of papillomas per mouse at 29 weeks were 1/40 (2%) and 0 for mice treated with the solvent, 10/40 (25%) and 0.3 for mice treated with a total of 10 mg ethyl carbamate, 19/40 (47%) and 3.4 for mice treated with a total of 120 mg ethyl carbamate and 23/35 (66%) and 4.5 for mice treated with a total of 10 mg vinyl carbamate. The incidence of skin papillomas in each of the treated groups was significantly greater than that in the control group [$P \leq 0.0035$; one-tailed Fisher's exact test]. The incidence of skin papillomas in the 10-mg vinyl carbamate-treated group was significantly greater than that in the approximately equimolar 10-mg ethyl carbamate-treated group [$P = 0.0004$; one-tailed Fisher's exact test]. The incidence of lung adenomas and the average number of lung adenomas per mouse at 32 weeks were 7/40 (17%) and 0.4 for mice treated with the solvent, 17/40 (42%) and 1.0 for mice treated with a total of 10 mg ethyl carbamate, 33/40 (82%) and 8.8 for mice treated with a total of 120 mg ethyl carbamate and 34/35 (97%) and 18.9 for mice treated with a total of 10 mg vinyl carbamate. The incidence of lung adenomas in each of the treated groups was significantly greater than that in the control group [$P \leq 0.0135$; one-tailed Fisher's exact test]. The incidence of lung adenomas in the 10-mg vinyl carbamate-treated

group was significantly greater than that in the approximately equimolar 10-mg ethyl carbamate-treated group [$P < 0.0001$; one-tailed Fisher's exact test] (Dahl *et al*., 1978).

In a second experiment, groups of 30–33 female CD-1 mice, 6–8 weeks of age, were treated topically on the shaved back with 1.2 mg croton oil in 200 μL redistilled acetone. Eighteen to 24 hour later, each mouse was treated topically with 2.5, 5 or 60 mg ethyl carbamate or 2.5 or 5 mg vinyl carbamate in 200 μL acetone or the solvent alone. The application of the carbamate compounds or solvent was repeated 1 week later. One week after the second application, all mice were treated twice weekly with 900 μg croton oil in 150 μL acetone. The experiment lasted 35 weeks, at which time ≥ 90% of the mice were still alive. The incidence of skin papillomas and the average number of papillomas per mouse at 32 weeks were 0/30 and 0 for mice treated with the solvent, 3/30 (10%) and 0.1 for mice treated with a total of 5 mg ethyl carbamate, 4/30 (13%) and 0.2 for mice treated with a total of 10 mg ethyl carbamate, 11/29 and 1.8 for mice treated with a total of 120 mg ethyl carbamate, 14/30 (38%) and 1.8 for mice treated with a total of 5 mg vinyl carbamate and 12/32 (37%) and 2.0 for mice treated with a total of 10 mg vinyl carbamate. The incidence of skin papillomas in the 120-mg ethyl carbamate-treated group and each of the vinyl carbamate-treated groups was significantly greater than that in the control group [$P \leq 0.0001$; one-tailed Fisher's exact test]. The incidence of skin papillomas in each of the vinyl carbamate-treated groups was significantly greater than that in the approximately equimolar ethyl carbamate-treated groups [$P \leq 0.0055$; one-tailed Fisher's exact test]. The incidence of lung adenomas and the average number of lung adenomas per mouse at 35 weeks were 15/27 (55%) and 0.9 for mice treated with the solvent, 13/28 (46%) and 0.9 for mice treated with a total of 5 mg ethyl carbamate, 16/30 (53%) and 1.0 for mice treated with a total of 10 mg ethyl carbamate, 24/29 (83%) and 7.3 for mice treated with a total of 120 mg ethyl carbamate, 27/30 (90%) and 4.5 for mice treated with a total of 5 mg vinyl carbamate and 32/32 (100%) and 12.0 for mice treated with a total of 10 mg vinyl carbamate. The incidence of lung adenomas in the 120-mg ethyl carbamate-treated group and each of the vinyl carbamate-treated groups was significantly greater than that in the control group [$P \leq 0.0268$; one-tailed Fisher's exact test]. The incidence of lung adenomas in each of the vinyl carbamate-treated groups was significantly greater than that in the approximately equimolar ethyl carbamate-treated groups [$P \leq 0.0004$; one-tailed Fisher's exact test] (Dahl *et al*., 1978).

Groups of 30 female CD-1 mice, 6–8 weeks of age, were treated topically on the shaved back with 2.5 μg TPA [purity not specified] in 100 μL acetone. Eighteen to 24 hours later, the mice received 5.8 or 11.5 μmol vinyl carbamate [purity not specified] or 5.8 or 11.5 μmol vinyl carbamate epoxide [purity not specified] in 200 μL acetone that contained 15% dimethyl sulfoxide (DMSO). The application of the vinyl carbamate and vinyl carbamate epoxide was repeated at weekly intervals for a total of five applications. This was then followed by twice weekly topical applications of 2.5 μg TPA in 100 μL acetone. Control mice were administered the solvent and TPA only. The experiment was terminated 22 weeks after the first application of vinyl carbamate

and vinyl carbamate epoxide. At this time, 95–100% of the mice were still alive. The average number of papillomas per mouse (± SD), as determined by gross examination, was 6.5 ± 5.2 for 5.8 μmol vinyl carbamate-treated, 10.5 ± 8.4 for 11.5 μmol vinyl carbamate-treated, 13.3 ± 9.2 for 5.8 μmol vinyl carbamate epoxide-treated, 13.8 ± 9.0 for 11.5 μmol vinyl carbamate epoxide-treated and 0.1 ± 0.3 for the solvent control animals. The average number of papillomas per mouse was significantly greater in each of the treated groups compared with the control group [$P < 0.001$; one-way ANOVA followed by SNK test] and significantly greater in the 5.8-μmol vinyl carbamate epoxide-treated group compared with the 5.8-μmol vinyl carbamate-treated group [$P < 0.001$; one-way ANOVA followed by SNK test] (Park *et al*., 1993).

In a second experiment, groups of 30 female CD-1 mice, 6–8 weeks of age, were treated topically on the shaved back with 2.5 μg TPA in 100 μL acetone. Eighteen to 24 hours later, the mice received applications of 1.15 or 11.5 μmol vinyl carbamate or 1.15 or 11.5 μmol vinyl carbamate epoxide in 200 μL acetone that contained 15% DMSO. Beginning 1 week after the treatment with vinyl carbamate or vinyl carbamate epoxide, the mice received twice-weekly topical applications of 2.5 μg TPA in 100 μL acetone. Control mice were given the solvent or TPA only. The experiment ended 22 weeks after the first application of vinyl carbamate and vinyl carbamate epoxide. At this time, 97–100% of the mice were still alive. The incidence of papillomas and the average number of papillomas per mouse (± SD), as determined by gross examination, were 56% and 0.9 ± 1.1 for 1.15 μmol vinyl carbamate-treated, 98% and 7.8 ± 5.1 for 11.5 μmol vinyl carbamate-treated, 93% and 5.2 ± 3.5 for 1.15 μmol vinyl carbamate epoxide-treated, 100% and 9.8 ± 4.7 for 11.5 μmol vinyl carbamate epoxide-treated and 7% and 0.07 ± 0.2 for the solvent control animals. The incidence of papillomas was significantly greater in each of the treated groups compared with the controls [$P < 0.0001$; one-tailed Fisher's exact test] and significantly greater in the 1.15-μmol vinyl carbamate epoxide-treated group compared with the 1.15-μmol vinyl carbamate-treated group [$P = 0.0011$; one-tailed Fisher's exact test]. With the exception of the group treated with 1.15 μmol vinyl carbamate, the average number of papillomas per mouse was significantly greater in each of the treated groups compared with the controls [$P < 0.05$; one-way ANOVA followed by SNK test]. The average number of papillomas per mouse was significantly greater in the 1.15- and 11.5-μmol vinyl carbamate epoxide-treated groups compared with the 1.15- and 11.5-μmol vinyl carbamate-treated groups, respectively [$P \leq 0.027$; one-way ANOVA followed by SNK test] (Park *et al*., 1993).

In a third experiment, groups of 30 female CD-1 mice [age not specified] were treated topically on the shaved back once a week with vinyl carbamate or vinyl carbamate epoxide in 200 μL acetone that contained 15% DMSO at the following doses: 11.5 μmol at weeks 1 and 2, 5.7 μmol at weeks 3 and 4 and 3.8 μmol from weeks 5 to 32. The mice were kept for an additional 10 weeks after the last treatment. Control mice were given the solvent only. Survival was not indicated. Thirty-two weeks after the first application of vinyl carbamate and vinyl carbamate epoxide, the incidence of papillomas and the average number of papillomas per mouse (± SD), as determined by gross

examination, were 4% and 0.03 ± 0.2 for vinyl carbamate-treated, 96% and 4.6 ± 2.6 for vinyl carbamate epoxide-treated and 0% and 0.0 ± 0.0 for the solvent control animals. The incidence of papillomas [$P < 0.0001$; one-tailed Fisher's exact test] and the average number of papillomas per mouse [$P < 0.001$; one-way ANOVA followed by SNK] in the vinyl carbamate epoxide-treated group were significantly greater than those in both the vinyl carbamate-treated and control groups. Twelve mice that received vinyl carbamate epoxide also had epidermoid carcinomas compared with none in the vinyl carbamate-treated or solvent control groups, a difference that was significant [$P = 0.0001$; one-tailed Fisher's exact test]. Forty-two weeks after the first application of vinyl carbamate and vinyl carbamate epoxide, malignant tumours were detected in both groups (two mammary adenocarcinomas, one lymphoblastic lymphoma, one haemangioma and one epidermoid carcinoma in mice treated with vinyl carbamate and 18 epidermoid carcinomas, four keratoacanthomas, three squamous-cell fibrosarcomas and one thymic lymphoma in mice treated with vinyl carbamate epoxide). None of the control mice had malignant tumours (Park *et al*., 1993).

3.5.3 *Subcutaneous or intramuscular administration*

(a) Mouse

Weanling female albino mice [number not specified] were given a subcutaneous injection of 100 μL water containing 12 mg ethyl carbamate [purity not specified] or equimolar amounts of *N*-hydroxyethyl carbamate [purity not specified]. The treatment was repeated 4 days later. The treatment of the control group was not specified and the effect of treatment upon survival was not indicated. Five months after treatment, the mice were killed and adenomas on the surface of the lung were counted. The number sof lung adenomas observed grossly was 434 in 28 mice treated with ethyl carbamate, 159 in 35 mice administered *N*-hydroxyethyl carbamate and six in 30 control mice (Miller *et al*., 1960).

In a second experiment, weanling female albino mice [number not specified] were treated in a manner identical to that described for the previous experiment. Four and a half months after treatment, the mice were killed and adenomas on the surface of the lung were counted. The number of lung adenomas was 90 in 18 mice treated with ethyl carbamate, 30 in 20 mice administered *N*-hydroxyethyl carbamate and two in an unspecified number of control mice (Miller *et al*., 1960).

Newborn SWR/J mice [age, sex and number not specified], weighing 1.1–1.7 g, were given a single subcutaneous injection of 2 μmol/g bw ethyl carbamate [purity not specified] or *N*-hydroxyethyl carbamate (purified by redistillation) in 50 μL/g bw distilled water. The experiment lasted 10 weeks, at which time the incidence of lung adenomas was assessed. Histology was conducted on questionable tumours. No differences in body weights were observed. Survival was not specified and there was no control group. The mean number of adenomas per mouse (95% CI) was 2.3 (1.8–2.7) in

mice treated with 2 μmol/g bw ethyl carbamate and 0.4 (0.0–0.9) in mice treated with 2 μmol/g bw *N*-hydroxyethyl carbamate (Kaye & Trainin, 1966).

(b) Rat

Groups of 12 female Sprague-Dawley rats [age not specified] were given 10 weekly intramuscular injections in the left hind leg of 250 μL trioctanoin or 250 μL trioctanoin that contained 1.15 or 2.30 μmol vinyl carbamate [purity not specified] or vinyl carbamate epoxide [purity not specified]. At 17–18 months, the incidence of injection-site sarcomas and mammary gland tumours was determined. The incidence of injection-site sarcomas was 0/12 for the 1.15-μmol vinyl carbamate-treated group, 1/11 (9%) for the 1.15-μmol vinyl carbamate epoxide-treated group, 0/12 for the 2.30-μmol vinyl carbamate-treated group, 4/11 (36%) for the 2.30-μmol vinyl carbamate epoxide-treated group and 0/11 for the control group. The incidence of injection-site sarcomas was significantly increased in the 2.30-μmol vinyl carbamate epoxide-treated group compared with the 2.30-μmol vinyl carbamate-treated group and the control group [$P < 0.045$; one-tailed Fisher's exact test]. The incidence and total number of mammary gland tumours were 3/12 (25%) and six for the 1.15-μmol vinyl carbamate-treated group, 1/11 (9%) and three for the 1.15-μmol vinyl carbamate epoxide-treated group, 3/11 (27%) and eight for the 2.30-μmol vinyl carbamate-treated group, 6/11 (54%) and 16 for the 2.30-μmol vinyl carbamate epoxide-treated group and 3/11 (27%) and seven for the control group (Park *et al*., 1993).

3.5.4 *Intraperitoneal administration*

(a) Mouse

Groups of 18–30 male or female Swiss mice, 2–3 months of age, were administered a single intraperitoneal injection of 10 mg ethyl carbamate [0.11 mmol] or 11.8 mg *N*-hydroxyethyl carbamate [0.11 mmol] in saline [volume not specified], or 5 or 25 mg *N*-hydroxyethyl carbamate in distilled water [volume not specified]. A control group of 46 mice remained untreated. Four days after the initial treatment, all groups received twice weekly dermal applications of 5% croton oil in liquid paraffin [volume not specified]. The incidence and multiplicity of skin tumours were assessed after 20 and 40 weeks of croton oil application; those of lung tumours were assessed after 40 weeks of croton oil application. Histopathology was conducted on the lungs. Survival was ≥ 97% after 20 weeks of croton oil application and ≥ 80% after 40 weeks of croton oil application. After 20 weeks of croton oil application, the incidence and multiplicity (± SD) of skin tumours were 14/30 (47%) and 0.6 ± 0.1 for mice treated with 10 mg ethyl carbamate, 3/29 (10%) and 0.1 ± 0.05 for mice treated with 11.8 mg *N*-hydroxyethyl carbamate, 14/20 (70%) and 1.0 ± 0.2 for mice treated with 25 mg *N*-hydroxyethyl carbamate and 4/18 (22%) and 0.3 ± 0.1 for mice treated with 5 mg *N*-hydroxyethyl carbamate versus 3/45 (7%) and 0.07 ± 0.05 for mice treated with

croton oil only. The skin tumour incidence was significantly increased in mice treated with 10 mg ethyl carbamate or 25 mg *N*-hydroxyethyl carbamate compared with the croton oil control mice [$P \leq 0.0001$; one-tailed Fisher's exact test]. The tumour multiplicity was significantly increased in all treatment groups [$P < 0.001$; one-way ANOVA followed by SNK test], with the exception of the mice treated with 11.8 mg *N*-hydroxyethyl carbamate. The incidence [$P = 0.0034$; two-tailed Fisher's exact test] and multiplicity [$P < 0.001$; one-way ANOVA followed by SNK test] of skin tumours in mice treated with 10 mg ethyl carbamate were significantly greater than those in mice treated with 11.8 mg *N*-hydroxyethyl carbamate. After 40 weeks of croton oil application, the incidence and multiplicity (± SD) of skin tumours were 18/30 (60%) and 0.9 ± 0.2 for mice treated with 10 mg ethyl carbamate, 6/28 (21%) and 0.2 ± 0.1 for mice treated with 11.8 mg *N*-hydroxyethyl carbamate, 17/18 (95%) and 1.9 ± 0.2 for mice treated with 25 mg *N*-hydroxyethyl carbamate and 8/18 (44%) and 0.25 ± 0.05 for mice treated with 5 mg *N*-hydroxyethyl carbamate versus 11/44 (25%) and 0.4 ± 0.1 for mice treated with croton oil only. The incidence [$P \leq 0.0026$; one-tailed Fisher's exact test] and multiplicity [$P < 0.001$; one-way ANOVA followed by SNK test] of skin tumours were significantly increased in mice treated with 10 mg ethyl carbamate or 25 mg *N*-hydroxyethyl carbamate compared with the croton oil control mice. The incidence [$P = 0.0037$; two-tailed Fisher's exact test] and multiplicity [$P < 0.001$; one-way ANOVA followed by SNK test] of skin tumours in mice treated with 10 mg ethyl carbamate were significantly greater than those in mice treated with 11.8 mg *N*-hydroxyethyl carbamate. After 40 weeks of croton oil application, the incidence and multiplicity (± SD) of lung tumours were 23/26 (88%) and 2.8 ± 0.5 for mice treated with 10 mg ethyl carbamate, 5/26 (19%) and 0.3 ± 0.1 for mice treated with 11.8 mg *N*-hydroxyethyl carbamate, 11/18 (6%) and 0.8 ± 0.2 for mice treated with 25 mg *N*-hydroxyethyl carbamate and 5/18 (28%) and 0.4 ± 0.1 for mice treated with 5 mg *N*-hydroxyethyl carbamate versus 2/42 (5%) and 0.05 ± 0.03 for mice treated with croton oil only. The lung-tumour incidence was significantly increased in mice treated with 10 mg ethyl carbamate or 25 mg *N*-hydroxyethyl carbamate compared with the croton-oil control mice [$P < 0.0001$; one-tailed Fisher's exact test]. Lung tumour multiplicity was significantly increased in all treatment groups [$P < 0.001$; one-way ANOVA followed by SNK test]. The incidence [$P < 0.0001$; two-tailed Fisher's exact test] and multiplicity [$P < 0.001$; one-way ANOVA followed by SNK test] of lung tumours in mice treated with 10 mg ethyl carbamate were significantly greater than those in mice treated with 11.8 mg *N*-hydroxyethyl carbamate (Berenblum *et al*., 1959).

Groups of 20 female Holtzman mice, 10 weeks of age, received an intraperitoneal injection of 200 µL water that contained 15 mg ethyl carbamate [0.17 mmol; purity not specified] or 17.7 mg *N*-hydroxyethyl carbamate [0.17 mmol; purity not specified]. A second, identical injection was given 4 hours later. After 1 week, the backs of the mice were shaved and 300 µL acetone that contained 0.3% croton oil was applied topically once a week for 18 weeks. There was no control group. After 18 weeks, 12/19 (63%) surviving mice treated with ethyl carbamate had a total of 33 skin papillomas and 9/18

(50%) surviving mice treated with *N*-hydroxyethyl carbamate had a total of 25 skin papillomas. [The incidence did not differ between the groups; $P = 0.3175$, one-tailed Fisher's exact test.] The mice were killed after 22 weeks, at which time 11/19 (58%) surviving mice treated with ethyl carbamate had a total of 57 lung adenomas and eight of 18 surviving mice treated with *N*-hydroxyethyl carbamate had a total of 29 lung adenomas. [The incidence did not differ between the groups; $P = 0.3127$, one-tailed Fisher's exact test] (Miller *et al*., 1960).

Groups of 22–25 female weanling SWR/J mice, 9–10 weeks of age, were given a single intraperitoneal injection of 5 or 10 μmol/g bw ethyl carbamate [purity not stated] or *N*-hydroxyethyl carbamate (purified by redistillation) in distilled water. The ethyl carbamate was administered as a 5 or 10% or 5-mM solution; the *N*-hydroxyethyl carbamate was given as a 5-mM solution. Additional groups that received 10 μmol/g bw ethyl carbamate or *N*-hydroxyethyl carbamate were also given 50 μg/g bw 2-diethylaminoethyl-2,2-diphenylpentanoate hydrochloride (SKF-525A) [purity not specified] dissolved in distilled water at a concentration of 5 mg/mL. Controls received injections of the same volume of 0.9% saline. SKF-525A inhibits the conversion of *N*-hydroxyethyl carbamate to ethyl carbamate. The experiment lasted 10 weeks, at which time the incidence of lung adenomas was assessed. Histology was conducted on questionable tumours. There were no differences in body weights, and survival was ≥ 88%. The incidence of adenomas and the mean number of adenomas per survivor (95% CI) were 57% and 1.0 (0.5–1.6) in mice treated with 5 μmol/g bw ethyl carbamate, 27% and 0.4 (0.1–0.7) in mice treated with 5 μmol/g bw *N*-hydroxyethyl carbamate, 100% and 4.0 (2.9–5.1) in mice treated with 10 μmol/g bw ethyl carbamate, 75% and 1.9 (1.2–2.5) in mice treated with 10 μmol/g bw *N*-hydroxyethyl carbamate, 96% and 4.1 (3.0–5.1) in mice treated with 10 μmol/g bw ethyl carbamate and 50 μg/g bw SKF-525A and 62% and 0.6 (0.4–0.9) in mice treated with 10 μmol/g bw *N*-hydroxyethyl carbamate and 50 μg/g bw SKF-525A. The incidence of adenomas [$P = 0.0127$; two-tailed Fisher's exact test] and mean number of adenomas per survivor in mice treated with 10 μmol/g bw *N*-hydroxyethyl carbamate were significantly lower than those in mice treated with 10 μmol/g bw ethyl carbamate. The mean number of adenomas per survivor in mice treated with 10 μmol/g bw *N*-hydroxyethyl carbamate and 50 μg/g bw SKF-525A was significantly lower than that in mice treated with 10 μmol/g bw *N*-hydroxyethyl carbamate alone (Kaye & Trainin, 1966).

Groups of 40–42 female CD-1 mice, 6–8 weeks of age, were treated topically on the shaved back with 1.2 mg croton oil in 200 μL redistilled acetone. Eighteen to 24 hours later, each mouse received a single intraperitoneal injection of 65 μg/g bw ethyl carbamate (> 99% pure by gas chromatography) or vinyl carbamate (melting point, 53–54°C, purity verified by elemental analysis, MS, infrared and nuclear magnetic resonance spectroscopy) in 5 μL/g bw 0.87% saline or the solvent alone. An additional group received two intraperitoneal injections of 1.0 mg/g bw ethyl carbamate in 5 μL/g bw 0.9% saline at a 1-week interval. One week after the last application, all mice were treated topically twice a week with 900 μg croton oil in 150 μL acetone.

The experiment lasted 28 weeks, at which time ≥ 63% of the mice were still alive. All animals were subjected to gross necropsy. The lungs were fixed in formalin and adenomas on the surface (≥ 1 mm in diameter) were counted. Representative tumours were fixed, sectioned and stained with haematoxylin and eosin. The incidence and the average number of skin papillomas per mouse at 25 weeks were 1/41 (2%) and 0 for mice treated with the solvent, 5/41 (12%) and 0.2 for mice treated with 65 μg/g bw ethyl carbamate, 24/37 (65%) and 5.4 for mice treated with a total of 2 mg/g bw ethyl carbamate and 15/26 (58%) and 3.9 for mice treated with 65 μg/g bw vinyl carbamate. The incidence of skin papillomas in the 2-mg/g bw ethyl carbamate-treated group and the 65-μg/g bw vinyl carbamate-treated group was significantly greater than that in the control group [$P < 0.0001$; one-tailed Fisher's exact test]. The incidence of skin papillomas in the 65-μg/g bw vinyl carbamate-treated group was significantly greater than that in the approximately equimolar 65-μg/g bw ethyl carbamate-treated group [$P = 0.0001$; one-tailed Fisher's exact test]. The incidence and the average number of lung adenomas per mouse at 28 weeks were 4/41 (10%) and 0.2 for mice treated with the solvent, 14/39 (36%) and 0.6 for mice treated with 65 μg/g bw ethyl carbamate, 30/32 (94%) and 28.3 for mice treated with a total of 2 mg/g bw ethyl carbamate and 24/26 (93%) and 19.2 for mice treated with 65 μg/g bw vinyl carbamate. The incidence of lung adenomas in each of the treated groups was significantly greater than that in the control group [$P \leq 0.0051$; one-tailed Fisher's exact test]. The incidence of lung adenomas in the 65-μg/g bw vinyl carbamate-treated group was significantly greater than that in the approximately equimolar 65-μg/g bw ethyl carbamate-treated group [$P < 0.0001$; one-tailed Fisher's exact test] (Dahl *et al*., 1978).

In a second experiment, groups of 20 or 33 female A/Jax mice, 6–8 weeks of age, were given a single intraperitoneal injection of 32 or 65 μg/g bw ethyl carbamate or vinyl carbamate in 5 μL/g bw 0.9% saline or 500 μg/g bw ethyl carbamate in 5 μL 0.9% saline or the solvent alone. The experiment lasted 22 weeks. At this time, survival was ≥ 95% in all groups except for the 65-μg/g bw vinyl carbamate-treated group, in which survival was 65%. The incidence of lung adenomas and the average number of lung adenomas per mouse were 3/20 (15%) and 0.2 for mice treated with the solvent, 15/20 (75%) and 0.8 for mice treated with 32 μg/g bw ethyl carbamate, 17/20 (85%) and 1.7 for mice treated with 65 μg/g bw ethyl carbamate, 19/19 (100%) and 17.4 for mice treated with 500 μg/g bw ethyl carbamate, 33/33 (100%) and 42.3 for mice treated with 32 μg/g bw vinyl carbamate and 13/13 (100%) and 19.1 for mice treated with 65 μg/g bw vinyl carbamate. The incidence of lung adenomas in each of the treated groups was significantly greater than that in the control group [$P \leq 0.0002$; one-tailed Fisher's exact test]. The incidence of lung adenomas in the 32-μg/g bw vinyl carbamate-treated group was significantly greater than that in the approximately equimolar 32-μg/g bw ethyl carbamate-treated group [$P = 0.0054$; one-tailed Fisher's exact test] (Dahl *et al*., 1978).

In a third experiment, groups of 20 or 30 female A/Jax mice, 6–8 weeks of age, received a single intraperitoneal injection of 16, 32 or 65 μg/g bw vinyl carbamate in 5 μL/g bw 0.9% saline or the solvent alone. The experiment lasted 28 weeks. At this time,

survival was ≥ 85% in all groups except for the 65-μg/g bw vinyl carbamate-treated, in which survival was 27%. The incidence of lung adenomas and the average number of lung adenomas per mouse were 5/17 (29%) and 0.4 for mice treated with the solvent, 20/20 (100%) and 20.0 for mice treated with 16 μg/g bw vinyl carbamate, 19/19 (100%) and 35.2 for mice treated with 32 μg/g bw vinyl carbamate and 8/8 (100%) and 21.4 for mice treated with 65 μg/g bw vinyl carbamate. The incidence of lung adenomas in each of the treated groups was significantly greater than that in the control group [$P \leq 0.0012$; one-tailed Fisher's exact test] (Dahl *et al*., 1978).

In a fourth experiment, groups of nine to 20 female A/Jax mice, 6–8 weeks of age, were given five intraperitoneal injections of 10 μg/g bw ethyl carbamate, a single intraperitoneal injection of 500 μg/g bw ethyl carbamate, 10 intraperitoneal injections of 5 μg/g bw vinyl carbamate, five intraperitoneal injections of 10 μg/g bw vinyl carbamate or a single intraperitoneal injection of 16 μg/g bw vinyl carbamate. Multiple injections were given at weekly intervals. The compounds were dissolved in 5 μL/g bw 0.9% saline. The control group received 10 weekly injections of the solvent alone. The experiment lasted 20 weeks and all animals survived. The incidence and the average number of lung adenomas per mouse were 3/14 (21%) and 0.4 for mice treated with the solvent, 15/20 (75%) and 1.2 for mice treated with five injections of 10 μg/g bw ethyl carbamate, 9/9 (100%) and 19.3 for mice treated with a single injection of 500 μg/g bw ethyl carbamate, 19/19 (100%) and 25.2 for mice treated with 10 injections of 5 μg/g bw vinyl carbamate, 20/20 (100%) and 53.2 for mice treated with five injections of 10 μg/g bw vinyl carbamate and 20/20 (100%) and 25.2 for mice treated with a single injection of 16 μg/g bw vinyl carbamate. The incidence of lung adenomas in each of the treated groups was significantly greater than in the control group [$P \leq 0.0028$; one-tailed Fisher's exact test]. The incidence of lung adenomas in the mice that received five injections of 10 μg/g bw vinyl carbamate was significantly greater than that in mice that received five injections of approximately equimolar 10 μg/g bw ethyl carbamate [$P = 0.0236$; one-tailed Fisher's exact test] (Dahl *et al*., 1978).

Male and female C57BL/6J × C3H/HeJ F_1 mice (B6C3F_1 mice) [initial number not specified], 1 day of age, were administered eight twice-weekly intraperitoneal injections of 46, 91, 136 or 5625 nmol/g bw ethyl carbamate [purity not specified], 46, 91 or 136 nmol/g bw vinyl carbamate [purity not specified but assessed by melting-point, infrared spectroscopy, MS, high-performance liquid chromatography and GC] or the solvent (5 μL/g bw 0.9% saline). Most (> 90%) of the mice survived the treatment, and 18–25 mice of each sex from each group were weaned. The study was terminated when the mice were 15–16 months old. All animals were subjected to gross necropsy. All tumours were fixed, sectioned and stained with haematoxylin and eosin. The incidence and multiplicity (± SD) of liver tumours (hepatomas) in male and female mice were, respectively: 6/25 (24%) and 0.2 ± 0.4 and 0/24 and 0.0 ± 0.0 for mice that received the solvent; 14/25 (56%) and 0.8 ± 0.9 and 2/23 (9%) and 0.1 ± 0.3 for mice that received 46 nmol/g bw ethyl carbamate; 22/25 (88%) and 2.5 ± 1.4 and 6/22 (27%) and 0.4 ± 0.9 for mice that received 91 nmol/g bw ethyl carbamate; 22/25 (88%) and 2.5 ± 1.9 and

8/23 (35%) and 0.8 ± 1.6 for mice that received 136 nmol/g bw ethyl carbamate; 9/9 (100%) and 3.1 ± 1.4 and 7/10 (70%) and 4.8 ± 5.1 for mice that received 5625 nmol/g bw ethyl carbamate; 15/19 (79%) and 3.6 ± 3.2 and 16/19 (84%) and 5.9 ± 3.9 for mice that received 46 nmol/g bw vinyl carbamate; 13/14 (93%) and 7.9 ± 9.6 and 17/19 (89%) and 2.5 ± 1.6 for mice that received 91 nmol/g bw vinyl carbamate; and 14/18 (78%) and 6.6 ± 5.8 and 10/12 (83%) and 5.6 ± 6.0 for mice that received 136 nmol/g bw vinyl carbamate. All groups, except for female mice treated with 46 nmol/g bw ethyl carbamate, had an increased multiplicity of hepatomas compared with their respective control groups. Also, equimolar doses of vinyl carbamate increased tumour multiplicity compared with equimolar doses of ethyl carbamate. Thymic lymphomas were only observed with 5625 nmol/g bw ethyl carbamate and 91 and 136 nmol/g bw vinyl carbamate. The incidence in male and female mice was, respectively, 5/17 (29%) and 9/20 (45%) for mice that received 5625 nmol/g bw ethyl carbamate, 3/19 (16%) and 4/21 (19%) for mice that received 91 nmol/g bw vinyl carbamate and 9/23 (39%) and 6/19 (32%) for mice that received 136 nmol/g bw vinyl carbamate. The increased incidence of thymic lymphomas compared with the respective control groups was significant in each of these groups, with the exception of male mice treated with 91 nmol/g bw vinyl carbamate. The incidence of thymic lymphomas in male and female mice treated with 136 nmol/g bw vinyl carbamate and female mice treated with 91 nmol/g bw vinyl carbamate was also significantly greater than that in the respective groups treated with an equimolar dose of ethyl carbamate. The incidence of lung adenomas in male and female mice was, respectively: 1/25 (4%) and 0/25 for mice that received the solvent; 0/25 and 2/24 (8%) for mice that received 46 nmol/g bw ethyl carbamate; 4/25 (16%) and 4/22 (22%) for mice that received 91 nmol/g bw ethyl carbamate; 2/25 (8%) and 6/23 (26%) for mice that received 136 nmol/g bw ethyl carbamate; 5/17 (29%) and 9/20 (45%) for mice that received 5625 nmol/g bw ethyl carbamate; 10/19 (53%) and 15/19 (79%) for mice that received 46 nmol/g bw vinyl carbamate; 15/19 (79%) and 16/21 (76%) for mice that received 91 nmol/g bw vinyl carbamate; and 10/23 (43%) and 10/19 (53%) for mice that received 136 nmol/g bw vinyl carbamate. All groups treated with vinyl carbamate (males and females combined) and the group treated with 5625 nmol/g bw ethyl carbamate had an increased incidence of lung adenomas compared with the control group. Also, equimolar doses of vinyl carbamate increased lung tumour incidence compared with equimolar doses of ethyl carbamate. The incidence of Harderian gland tumours in male and female mice was, respectively: 0/25 and 0/25 for mice that received the solvent; 0/25 and 1/24 (4%) for mice that received 46 nmol/g bw ethyl carbamate; 0/25 and 0/22 for mice that received 91 nmol/g bw ethyl carbamate; 2/25 (8%) and 3/23 (9%) for mice that received 136 nmol/g bw ethyl carbamate; 3/17 (18%) and 3/20 (15%) for mice that received 5625 nmol/g bw ethyl carbamate; 4/19 (21%) and 6/19 (32%) for mice that received 46 nmol/g bw vinyl carbamate; 0/19 and 5/21 (24%) for mice that received 91 nmol/g bw vinyl carbamate; and 1/23 (4%) and 4/19 (21%) for mice that received 136 nmol/g bw vinyl carbamate. Only female mice treated with vinyl carbamate and the male mice treated with 46 nmol/g bw vinyl carbamate had an

increased incidence of Harderian gland tumours compared with their respective control groups. Also, male and female mice treated with 46 nmol/g bw vinyl carbamate and female mice treated with 91 nmol/g bw vinyl carbamate had an increased Harderian gland tumour incidence compared with the respective groups treated with equimolar doses of ethyl carbamate (Dahl *et al*., 1980).

In a second experiment, groups of 30 female A/J mice, 6–8 weeks of age, received a single intraperitoneal injection of 3 or 6 μmol/g bw [ethyl-$^{1}H_5$]ethyl carbamate or [ethyl-$^{2}H_5$]ethyl carbamate (melting-point, 46–47 °C, satisfactory elemental analysis, mass spectrum) or the solvent (5 μL/g bw 0.9% saline). The experiment ended 5 months later, at which time most (≥ 87%) of the mice were still alive. The incidence and multiplicity (± SD) of lung adenomas were 8/30 (27%) and 0.3 ± 0.1 for mice that received the solvent; 30/30 (100%) and 5.3 ± 2.4 for mice that received 3 μmol/g bw [ethyl-$^{1}H_5$] ethyl carbamate; 26/26 (100%) and 4.7 ± 2.6 for mice that received 3 μmol/g bw [ethyl-$^{2}H_5$]ethyl carbamate; 29/29 (100%) and 10.9 ± 6.8 for mice that received 6 μmol/g bw [ethyl-$^{1}H_5$]ethyl carbamate; and 30/30 (100%) and 9.6 ± 4.4 for mice that received 6 μmol/g bw [ethyl-$^{2}H_5$]ethyl carbamate. The tumour multiplicity in mice that received [ethyl-$^{1}H_5$]ethyl carbamate did not differ statistically from that observed in mice that received equimolar doses of [ethyl-$^{2}H_5$]ethyl carbamate (Dahl *et al*., 1980).

In a third experiment, a group of 17–20 female A/J mice, 6–8 weeks of age, were administered a single intraperitoneal injection of 4000 nmol/g bw ethyl carbamate, 4000 nmol/g bw *N*-hydroxyethyl carbamate [purity not specified], 150 nmol/g bw vinyl carbamate or the solvent (5 μL/g bw 0.9% saline). Additional groups were pretreated immediately before injection with the carbamate test compounds with intraperitoneal injections of 40 nmol/g bw 2-(2,4-dichloro-6-phenyl)phenoxyethylamine (DPEA), an inhibitor of cytochrome-P450 (CYP). Mice in some of the DPEA-treated groups received seven additional intraperitoneal injections of DPEA at 2-hour intervals. The experiment was terminated 7 months later, at which time most of the mice were still alive. The incidence and multiplicity (± SD) of lung adenomas were 2/19 (10%) and 0.1 ± 0.3 for mice that received the solvent, 18/18 (100%) and 7.1 ± 3.7 for mice that received 4000 nmol/g bw ethyl carbamate, 17/19 (89%) and 4.0 ± 2.3 for mice that received 4000 nmol/g bw *N*-hydroxyethyl carbamate, and 15/15 (100%) and 11.3 ± 3.4 for mice that received 150 nmol/g bw vinyl carbamate. Treatment with a total dose of 320 nmol/g bw DPEA significantly decreased the tumour multiplicity in mice that received 4000 nmol/g bw *N*-hydroxyethyl carbamate (2.4 ± 1.6 versus 4.0 ± 2.3) (Dahl *et al*., 1980).

In a fourth experiment, groups of 10, 15 or 20 female A/J mice, 6–8 weeks of age, received a single intraperitoneal injection of 1120 or 5620 nmol/g bw ethyl carbamate, 950 or 4760 nmol/g bw *N*-hydroxyethyl carbamate, 57 or 115 nmol/g bw vinyl carbamate or the solvent (5 μL/g bw 0.9% saline). The experiment was terminated 6.5 months later, at which time most (> 90%) of the mice were still alive. The incidence and multiplicity (± SD) of lung adenomas were 7/15 (47%) and 0.7 ± 0.1 for mice that received the solvent, 14/15 (93%) and 3.7 ± 2.4 for mice that received 1120 nmol/g

bw ethyl carbamate, 15/15 (100%) and 17.9 ± 4.3 for mice that received 5620 nmol/g bw ethyl carbamate, 12/15 (80%) and 1.5 ± 1.0 for mice that received 950 nmol/g bw *N*-hydroxyethyl carbamate, 14/14 (100%) and 7.8 ± 3.8 for mice that received 4760 nmol/g bw *N*-hydroxyethyl carbamate, 9/10 (90%) and 3.7 ± 3.6 for mice that received 57 nmol/g bw vinyl carbamate, and 14/15 (93%) and 6.4 ± 3.1 for mice that received 115 nmol/g bw vinyl carbamate (Dahl *et al*., 1980).

In a fifth experiment, groups of 13–20 female A/J mice, 6–8 weeks of age, were given a single intraperitoneal injection of 2000 or 4000 nmol/g bw ethyl carbamate or *N*-hydroxyethyl carbamate, 75 or 150 nmol/g bw vinyl carbamate or the solvent (5 μL/g bw 0.9% saline). The experiment was terminated 6.5 months later, at which time most (> 80%) of the mice were still alive. The incidence and multiplicity (± SD) of lung adenomas were 7/16 (44%) and 0.7 ± 0.1 for mice that received the solvent, 15/15 (100%) and 4.3 ± 2.1 for mice that received 2000 nmol/g bw ethyl carbamate, 14/14 (100%) and 9.5 ± 3.6 for mice that received 4000 nmol/g bw ethyl carbamate, 10/15 (67%) and 1.1 ± 1.1 for mice that received 2000 nmol/g bw *N*-hydroxyethyl carbamate, 18/19 (95%) and 3.2 ± 2.2 for mice that received 4000 nmol/g bw *N*-hydroxyethyl carbamate, 19/19 (100%) and 3.8 ± 2.2 for mice that received 75 nmol/g bw vinyl carbamate, and 19/19 (100%) and 12.1 ± 4.0 for mice that received 150 nmol/g bw vinyl carbamate. Tumour multiplicity in mice treated with ethyl carbamate was significantly higher than that in mice treated with equimolar doses of *N*-hydroxyethyl carbamate [$P \leq 0.002$; one-way ANOVA followed by SNK test] (Dahl *et al*., 1980).

A study was conducted to determine whether vinyl carbamate showed the same strain-specific tumorigenicity patterns as ethyl carbamate. Specifically, groups of male and female A/J, C3HeB/FeJ (C3H) and C57BL/6J mice, 3–5 months of age, received single intraperitoneal injections of 100 μL 0.9% saline solution that contained 30, 100, 300 and 1000 mg/kg bw ethyl carbamate (≥ 99% pure) or 1, 3, 10, 30 and 60 mg/kg bw vinyl carbamate (≥ 99% pure). Two control groups, one untreated and the other injected with 100 μL 0.9% saline were available. The groups comprised 32 mice (16 males and 16 females), except for the C3H and C57BL/6J groups treated with 60 mg/kg bw vinyl carbamate, which comprised 16 mice (eight males and eight females). All animals were killed 24 weeks after the injection. At the end of the experiment, 26–32 mice were alive in each of the groups (14 and 16, respectively, in the C3H and C57BL/6J groups treated with 60 mg/kg bw vinyl carbamate). Only mice that survived to the end of the experiment were used to assess the extent of tumorigenicity. The incidence of lung tumours was determined by gross examination of the lungs using a dissecting microscope. The incidence and multiplicity (± SD) of lung tumours in A/J mice were: untreated control, 25% and 0.3 ± 0.54 tumours/mouse; 0.9% saline control, 28% and 0.4 ± 0.71 tumours/mouse; 30-mg/kg ethyl carbamate-treated, 71% and 0.9 ± 0.75 tumours/mouse; 100-mg/kg ethyl carbamate-treated, 94% and 1.7 ± 0.96 tumours/mouse; 300-mg/kg ethyl carbamate-treated, 100% and 7.3 ± 2.86 tumours/mouse; 1000-mg/kg ethyl carbamate-treated, 100% and 29.5 ± 7.67 tumours/mouse; 1-mg/kg vinyl carbamate-treated, 33% and 0.4 ± 0.68 tumours/mouse; 3-mg/kg vinyl carbamate-treated, 81% and 1.4 ± 1.08

tumours/mouse; 10-mg/kg vinyl carbamate-treated, 100% and 7.2 ± 4.16 tumours/mouse; 30-mg/kg vinyl carbamate-treated, 100% and 43.0 ± 12.33 tumours/mouse; and 60-mg/kg vinyl carbamate-treated, 100% and 40.2 ± 14.07 tumours/mouse. The incidence and multiplicity (± SD) of lung tumours in C3H mice were: untreated control, 3% and 0.0 ± 0.19 tumours/mouse; 0.9% saline control, 3% and 0.0 ± 0.17 tumours/mouse; 30-mg/kg ethyl carbamate-treated, 3% and 0.0 ± 0.19 tumours/mouse; 100-mg/kg ethyl carbamate-treated, 6% and 0.1 ± 0.25 tumours/mouse; 300-mg/kg ethyl carbamate-treated, 14% and 0.2 ± 0.47 tumours/mouse; 1000-mg/kg ethyl carbamate-treated, 23% and 0.3 ± 0.70 tumours/mouse; 1-mg/kg vinyl carbamate-treated, 0% and 0.0 ± 0.00 tumours/mouse; 3-mg/kg vinyl carbamate-treated, 0% and 0.0 ± 0.00 tumours/mouse; 10-mg/kg vinyl carbamate-treated, 20% and 0.4 ± 1.00 tumours/mouse; 30-mg/kg vinyl carbamate-treated, 47% and 0.8 ± 1.06 tumours/mouse; and 60-mg/kg vinyl carbamate-treated, 43% and 0.6 ± 0.76 tumours/mouse). The incidence and multiplicity (± SD) for lung tumours in C57BL/6J mice were: untreated control, 6% and 0.1 ± 0.25 tumours/mouse; 0.9% saline control, 3% and 0.0 ± 0.18 tumours/mouse; 30-mg/kg ethyl carbamate-treated, 13% and 0.1 ± 0.34 tumours/mouse; 100-mg/kg ethyl carbamate-treated, 13% and 0.1 ± 0.34 tumours/mouse; 300-mg/kg ethyl carbamate-treated, 23% and 0.3 ± 0.71 tumours/mouse; 1000-mg/kg ethyl carbamate-treated, 66% and 1.2 ± 1.39 tumours/mouse; 1-mg/kg vinyl carbamate-treated, 7% and 0.1 ± 0.40 tumours/mouse; 3-mg/kg vinyl carbamate-treated, 13% and 0.1 ± 0.34 tumours/mouse; 10-mg/kg vinyl carbamate-treated, 9% and 0.1 ± 0.42 tumours/mouse; 30-mg/kg vinyl carbamate-treated, 78% and 1.7 ± 1.53 tumours/mouse; and 60-mg/kg vinyl carbamate-treated, 100% and 6.1 ± 2.91 tumours/mouse. Lung-tumour incidence was significantly greater than that in the 0.9% saline control group in A/J mice with all doses of ethyl carbamate and ≥ 3 mg/kg vinyl carbamate, in C3H mice with doses of 1000 mg/kg ethyl carbamate and ≥ 10 mg/kg vinyl carbamate and in C57BL/6J mice with doses of ≥ 300 mg/kg ethyl carbamate and ≥ 30 mg/kg vinyl carbamate [$P \leq 0.04$; one-tailed Fisher's exact test]. In all three strains, lung tumour incidence with 30 mg/kg vinyl carbamate was significantly greater than that with the approximately equimolar dose of 30 mg/kg ethyl carbamate [$P \leq 0.001$; one-tailed Fisher's exact test]. Lung tumour multiplicity was significantly greater than that in the 0.9% saline control group in A/J mice with doses of ≥ 300 mg/kg ethyl carbamate and ≥ 10 mg/kg vinyl carbamate, in C3H mice with doses of ≥ 10 mg/kg vinyl carbamate and in C57BL/6 mice with doses of 1000 mg/kg ethyl carbamate and ≥ 30 mg/kg vinyl carbamate [$P < 0.05$; one-way ANOVA, followed by Dunnett's test, respectively]. In all three strains, lung tumour multiplicity with 30 mg/kg vinyl carbamate was significantly greater than that with the approximately equimolar dose of 30 mg/kg ethyl carbamate [$P < 0.0001$; one-way ANOVA followed by SNK test] (Allen *et al*., 1986).

Groups of male A/J mice [number not specified], 6 weeks of age, were administered a single intraperitoneal injection of 60 mg/kg bw vinyl carbamate [purity not specified] in 100 µL tricaprylin or the solvent alone. Interim killings were performed at 7, 8, 10, 12 and 14 months of age. The overall survival was not specified. Lungs were fixed

and examined histologically. The number of mice examined and the mean number of lung lesions (hyperplasias, adenomas and/or carcinomas) per mouse (± standard error [SE]) were four and 0.00 ± 0.00 for control mice and nine and 36.89 ± 4.46 for vinyl carbamate-treated mice killed at 7 months of age, five and 0.00 ± 0.00 for control and 12 and 31.25 ± 2.90 for vinyl carbamate-treated mice killed at 8 months of age, 11 and 36.73 ± 1.93 for vinyl carbamate-treated mice killed at 10 months of age (no control mice were sacrified at 10 months), 19 and 0.58 ± 0.14 for control and eight and 39.50 ± 3.58 for vinyl carbamate-treated mice killed at 12 months of age, 10 and 0.80 ± 0.33 for control and 44 and 37.34 ± 1.06 for vinyl carbamate-treated mice killed at 14 months of age. At each time-point (for which control animals were available), the number of lesions per mouse was significantly greater in the vinyl carbamate-treated animals [$P < 0.001$; Student's *t*-test] compared with the control group. At 7, 8, 10, 12 and 14 months, hyperplasias accounted for 32%, 8%, 2%, 2% and ~0%, respectively, of the lesions in the vinyl carbamate-treated mice, the relative contribution of adenomas was 66%, ~90%, ~82%, ~52% and 45%, respectively, and the relative contribution of carcinomas was 2%, 2%, ~16%, ~46% and 55%, respectively (Foley *et al*., 1991).

A group of 55 male and 50 female C57Bl/10J mice, 4–6 weeks of age, received intraperitoneal injections of 6 mg/kg bw vinyl carbamate (purity, > 99%) in 10 µL/g bw sterile physiological saline once a week for 35 weeks. A group of 10 male and 10 female control mice remained untreated. Five vinyl carbamate-treated mice of each sex were killed at 5 weeks; the remaining mice formed the main body of the study. Male mice treated with vinyl carbamate weighed significantly less than control males beginning at week 14, and weighed 76% of the control males by 57 weeks. The body weight of the female mice was not affected by treatment with vinyl carbamate. There were few unscheduled early deaths during the 35-week treatment period; however, ~70% of the mice either died or were removed due to morbidity by the time the experiment was terminated at week 59. Gross necropsy was performed and histopathology was conducted. Treatment with vinyl carbamate resulted in the formation of hepatocellular adenomas (2/49 (4%) males and 1/45 (2%) females), hepatocellular carcinomas (8/49 (16%) males and 9/45 (20%) females), liver haemangiosarcomas (30/49 (6%) males and 25/45 (56%) females), liver haemangiomas (31/49 (63%) males and 24/45 (53%) females) and liver histiocytic sarcomas (6/49 (12%) males and 1/45 (2%) females). The incidence of liver haemangiosarcoma and liver hemangioma was significantly increased in both sexes compared with the control group [$P \leq 0.0015$; one-tailed Fisher's exact test] (Wright *et al*., 1991).

Groups of 30–50 female A/Jax mice, 6–8 weeks of age, received a single intraperitoneal injection of 5 µL/g bw trioctanoin or 5 µL/g bw trioctanoin that contained 34 or 68 nmol/g bw vinyl carbamate [purity not specified] or vinyl carbamate epoxide [purity not specified]. At 6 months, the mice were killed, the lungs were fixed in buffered formalin and the number of adenomas (> 1 mm in diameter) was determined. The number of mice that survived to the end of the experiment was 30/30 for the 34-nmol/g bw vinyl carbamate-treated group, 19/30 for the 34-nmol/g bw vinyl

carbamate epoxide-treated group, 30/30 for the 68-nmol/g bw vinyl carbamate-treated group, 15/50 for the 68-nmol/g bw vinyl carbamate epoxide-treated and 28/30 for the solvent-treated control group. The incidence of lung adenomas and the average number of lung adenomas per mouse (± SD) were 26/30 (87%) and 2.0 ± 1.4 for the 34-nmol/g bw vinyl carbamate-treated group, 16/19 (84%) and 1.4 ± 1.9 for the 34-nmol/g bw vinyl carbamate epoxide-treated group, 30/30 (100%) and 4.4 ± 2.5 for the 68-nmol/g bw vinyl carbamate-treated group, 13/15 (87%) and 3.8 ± 2.8 for the 68-nmol/g bw vinyl carbamate epoxide-treated group and 9/28 (32%) and 0.3 ± 0.5 for the solvent-treated control group. The incidence of lung adenomas in each of the treated groups was significantly greater than that in the control group [$P \leq 0.0007$; one-tailed Fisher's exact test]. The average number of lung adenomas per mouse was greater in the groups treated with 68 nmol/g bw vinyl carbamate and vinyl carbamate epoxide than in the control group [$P \leq 0.001$; one-way ANOVA followed by SNK test] (Park *et al*., 1993).

In a second study, groups of 26–29 male B6C3F1 mice, 12 days of age, received a single intraperitoneal injection of 10 µL trioctanoin or 10 µL/g bw trioctanoin that contained 1400 nmol/g bw ethyl carbamate, 29 nmol/g bw vinyl carbamate or 4.8, 12 or 24 nmol/g bw vinyl carbamate epoxide. At 9 months of age, the mice were killed and the number of hepatomas (> 2 mm in diameter and visible on the surface) were determined. The number of mice that survived to the end of the experiment was 28/28 for the 1400-nmol/g bw ethyl carbamate-treated, 29/29 for the 29 nmol/g bw vinyl carbamate-treated, 29/29 for the 4.8-nmol/g bw vinyl carbamate epoxide-treated, 5/27 for the 12-nmol/g bw vinyl carbamate epoxide-treated, 4/26 for the 24-nmol/g bw vinyl carbamate epoxide-treated and 29/29 for the solvent-treated control animals. The incidence of hepatomas and the average number of hepatomas per mouse (± SD) were 100% and 12.1 ± 3.5 for the 1400-nmol/g bw ethyl carbamate-treated group, 96% and 11.3 ± 5.0 for the 29-nmol/g bw vinyl carbamate-treated group, 28% and 0.4 ± 0.9 for the 4.8-nmol/g bw vinyl carbamate epoxide-treated group, 60% and 8.8 ± 9.1 for the 12-nmol/g bw vinyl carbamate epoxide-treated group, 100% and 49.0 ± 5.4 for the 24-nmol/g bw vinyl carbamate epoxide-treated group and 10% and 0.1 ± 0.3 for the solvent-treated control group. With the exception of the 4.8-nmol/g bw vinyl carbamate epoxide-treated group, the incidence of hepatomas [$P \leq 0.03$; one-tailed Fisher's exact test] and the average number of hepatomas per mouse [$P < 0.05$; one-way ANOVA followed by Dunnett's test] were greater in each of the treatment groups compared with the control group (Park *et al*., 1993).

Groups of 25 male NIH strain A mice, 6 weeks of age, were given single intraperitoneal injections of 10 mL/kg bw isotonic saline alone or containing 1.12, 4.6 or 11.2 mmol/kg bw 2-hydroxyethyl carbamate (purity not stated but assessed by melting-point, GC, nuclear magnetic resonance spectroscopy and MS) or 1.12 or 4.6 mmol/kg bw ethyl carbamate [purity not stated]. The mice were maintained for 16 weeks after the injection, at which time the incidence and multiplicity of lung adenomas was assessed. The incidence of lung adenomas (> 1 mm) was determined by gross examination using a dissecting microscope; representative tumours were sectioned

and examined histologically. With the exception of one mouse in the 4.6-mmol ethyl carbamate-treated group, all mice survived to the end of the experiment. No tumours were observed grossly outside of the lungs. The incidence and multiplicity (± SE) of lung adenomas were: 4/25 (16%) and 0.16 ± 0.07 tumours/mouse for the 1.12-mmol/kg bw 2-hydroxyethyl carbamate-treated group; 7/25 (28%) and 0.32 ± 0.11 tumours/mouse for the 4.6-mmol/kg bw 2-hydroxyethyl carbamate-treated group; 7/25 (28%) and 0.32 ± 0.11 tumours/mouse for the 11.2-mmol/kg bw 2-hydroxyethyl carbamate-treated group; 23/25 (92%) and 3.3 ± 0.3 tumours/mouse for the 1.12-mmol/kg bw ethyl carbamate-treated group; and 24/24 (100%) and 13.5 ± 0.8 tumours/mouse for the 4.6-mmol/kg bw ethyl carbamate-treated group; versus 1/25 (4%) and 0.04 ± 0.04 tumours/mouse for the control group. The incidence in each of the treated groups was significantly greater than that in the control group. The tumour multiplicity in the groups treated with ethyl carbamate was significantly greater than that in the control group. The incidence [$P < 0.0001$; two-tailed Fisher's exact test] and multiplicity [$P < 0.001$; one-way ANOVA followed by SNK test] in the ethyl carbamate-treated groups were significantly greater than those in the respective 2-hydroxyethyl carbamate-treated groups (Mirvish *et al*., 1994).

Male and female C57BL/6J × BALB/cJ mice ($B6CF_1$) [number not specified], 15 days of age, were administered a single intraperitoneal injection of 30 nmol/kg bw vinyl carbamate [purity not specified] in saline [volume not specified]. Subgroups of mice were killed at selected intervals from 30 to 122 weeks of age. Overall survival was not specified. Lungs were examined histologically. In those killed at 6–12 months of age, the number of mice examined, the percentage incidence of lung tumours (alveolar/bronchiolar adenomas or carcinomas) and number of tumours per mouse were: three, 0% and none for male control mice; three, 0% and none for female control mice; six, 0% and none for male vinyl carbamate-treated mice; and three, 0% and none for female vinyl carbamate-treated mice. For those killed at 12–18 months of age, the values were: 10, 30% and 0.40 for male control mice, 10, 10% and 0.20 for female control mice; 15, 33% and 0.40 for male vinyl carbamate-treated mice; and 15, 40% and 0.67 for female vinyl carbamate-treated mice. For those killed at 18–24 months of age, the values were: 27, 22% and 0.30 for male control mice; 47, 13% and 0.13 for female control mice; 65, 46% and 0.71 for male vinyl carbamate-treated mice; and 111, 45% and 0.76 for female vinyl carbamate-treated mice. The incidence of lung tumours was significantly greater in male and female vinyl carbamate-treated mice than in male and female control mice [$P = 0.0264$ and 0.0001, respectively; one-tailed Fisher's exact test]. For those killed at > 24 months of age, the values were: 42, 50% and 0.64 for male control mice; 45, 27% and 0.47 for female control mice; and 20, 45% and 1.0 for male vinyl carbamate-treated mice. For the entire experiment, the values were: 82, 37% and 0.48 for male control mice; 105, 18% and 0.28 for female control mice; 106, 41% and 0.68 for male vinyl carbamate-treated mice; and 129, 43% and 0.73 for female vinyl carbamate-treated mice. The incidence of lung tumours was significantly greater in female vinyl carbamate-

treated mice compared with female control mice [$P = 0.0001$; one-tailed Fisher's exact test] (Massey *et al*., 1995).

An experiment was conducted with $CB6F_1$-Tg *HRAS2* mice (*HRAS2* mice), a hemizygous transgenic mouse strain that carries the human prototype *c-Ha-RAS* gene, and their non-transgenic (non-Tg) littermates. Groups of 31 male and 29 female *HRAS2* and 31 male and 31 female non-Tg mice, 7 weeks of age, received a single intraperitoneal injection of 60 mg/kg bw vinyl carbamate [purity not specified] in 10 mL/kg bw sterile 0.9% saline. Control groups consisting of 10 male and 10 female *HRAS2* and 10 male and 10 female non-Tg mice received a single injection of the solvent. The experiment lasted 16 weeks. Nine male and nine female *HRAS2* mice that were treated with vinyl carbamate died before the end of the experiment. Mean body weights of both sexes of non-Tg mice treated with vinyl carbamate were significantly lower than their respective control non-Tg mice. Complete necropsy was performed. Target tissues (forestomach, lung and spleen) and any gross lesions were examined histopathologically. Statistical comparisons of differences in incidence and multiplicity between *HRAS2* and non-Tg mice were conducted using the one-tailed Fisher's exact test and Student's *t*-test, respectively. The percentage of mice killed 16 weeks after treatment with lung adenomas and the mean number of adenomas (± SD)/mouse were 100% and 14.76 ± 5.36 for male vinyl carbamate-treated *HRAS2* mice, 10.0% and 0.10 ± 0.32 for male solvent-treated *HRAS2* mice, 88.5% and 2.92 ± 2.10 for male vinyl carbamate-treated non-Tg mice, 0% and 0.0 ± 0.0 for male solvent-treated non-Tg mice, 100% and 20.53 ± 7.54 for female vinyl carbamate-treated *HRAS2* mice, 0% and 0.0 ± 0.0 for female solvent-treated *HRAS2* mice, 96.2% and 3.19 ± 1.55 for female vinyl carbamate-treated non-Tg mice and 0% and 0.0 ± 0.0 for female solvent-treated non-Tg mice. In both male and female *HRAS2* and non-Tg mice, the incidence of lung adenomas [$P < 0.0001$] and the mean number of adenomas/mouse [$P < 0.001$] were significantly greater in the mice treated with vinyl carbamate than in their respective control groups. In both male and female *HRAS2* mice treated with vinyl carbamate, the mean number of adenomas/mouse was significantly greater than that in male and female non-Tg mice treated with vinyl carbamate. The percentage of mice with lung carcinomas and the mean number of carcinomas (± SD)/mouse were 47.1% and 0.65 ± 0.79 for male vinyl carbamate-treated *HRAS2* mice, 0% and 0.0 ± 0.0 for male solvent-treated *HRAS2* mice, 3.9% and 0.04 ± 0.20 for male vinyl carbamate-treated non-Tg mice, 0% and 0.0 ± 0.0 for male solvent-treated non-Tg mice, 53.3% and 0.67 ± 0.72 for female vinyl carbamate-treated *HRAS2* mice, 0% and 0.0 ± 0.0 for female solvent-treated *HRAS2* mice, 0% and 0.0 ± 0.0 for female vinyl carbamate-treated non-Tg mice and 0% and 0.0 ± 0.0 for female solvent-treated non-Tg mice. In both male and female *HRAS2* mice, the incidence of lung carcinomas [$P \leq 0.01$] and the mean number of carcinomas/mouse [$P \leq 0.015$] were significantly greater in the mice treated with vinyl carbamate than in their respective control groups. In both male and female *HRAS2* mice treated with vinyl carbamate, the incidence of carcinomas and the mean number of carcinomas/mouse were significantly greater than those in male and

female non-Tg mice treated with vinyl carbamate. The percentage of mice with lung adenomas and carcinomas, and the mean number of adenomas and carcinomas (± SD)/mouse were 100% and 15.41 ± 5.43 for male vinyl carbamate-treated *HRAS2* mice, 10.0% and 0.10 ± 0.32 for male solvent-treated *HRAS2* mice, 88.5% and 2.96 ± 2.18 for male vinyl carbamate-treated non-Tg mice, 0% and 0.0 ± 0.0 for male solvent-treated non-Tg mice, 100% and 21.20 ± 7.59 for female vinyl carbamate-treated *HRAS2* mice, 0% and 0.0 ± 0.0 for female solvent-treated *HRAS2* mice, 96.2% and 3.19 ± 1.55 for female vinyl carbamate-treated non-Tg mice and 0% and 0.0 ± 0.0 for female solvent-treated non-Tg mice. In both male and female *HRAS2* and non-Tg mice, the incidence of adenomas and carcinomas [$P < 0.0001$] and the mean number of adenomas and carcinomas/mouse [$P < 0.001$] were significantly greater in the mice treated with vinyl carbamate compared with their respective controls. In both male and female *HRAS2* mice treated with vinyl carbamate, the mean number of adenomas and carcinomas/mouse was significantly greater than that in the male and female non-Tg mice treated with vinyl carbamate. The percentage of mice with spleen haemangiosarcomas and the mean number of spleen haemangiosarcomas (± SD)/mouse were 91% and 2.88 ± 1.50 for male vinyl carbamate-treated *HRAS2* mice, 10% and 0.10 ± 0.32 for male solvent-treated *HRAS2* mice, 0% and 0.0 ± 0.0 for male vinyl carbamate-treated non-Tg mice, 0% and 0.0 ± 0.0 for male solvent-treated non-Tg mice, 86% and 2.13 ± 1.46 for female vinyl carbamate-treated *HRAS2* mice, 10% and 0.10 ± 0.32 for female solvent-treated *HRAS2* mice, 0% and 0.0 ± 0.0 for female vinyl carbamate-treated non-Tg mice and 0% and 0.0 ± 0.0 for female solvent-treated non-Tg mice. In both male and female *HRAS2* mice, the incidence of spleen haemangiosarcomas [$P < 0.0001$] and mean number of spleen haemangiosarcomas/mouse [$P < 0.001$] were significantly greater in the mice treated with vinyl carbamate than in their respective control groups. In both male and female *HRAS2* mice treated with vinyl carbamate, the mean number of spleen haemangiosarcomas/mouse and incidence of spleen haemangiosarcomas were significantly greater than those in male and female non-Tg mice treated with vinyl carbamate. The percentage of mice with lung haemangiosarcomas was 11.8% for male vinyl carbamate-treated *HRAS2* mice, 0% for male solvent-treated *HRAS2* mice, 0% for male vinyl carbamate-treated non-Tg mice, 0% for male solvent-treated non-Tg mice, 20.0% for female vinyl carbamate-treated *HRAS2* mice, 0% for female solvent-treated *HRAS2* mice, 0% for female vinyl carbamate-treated non-Tg mice and 0% for female solvent-treated non-Tg mice. In female *HRAS2* mice treated with vinyl carbamate, the incidence of lung haemangiosarcomas was significantly greater than that in female non-Tg mice treated with vinyl carbamate. Male *HRAS2* mice treated with vinyl carbamate had a 5% incidence of forestomach papillomas and a 14% incidence of forestomach squamous-cell carcinomas. Female *HRAS2* mice treated with vinyl carbamate had a 5% incidence of forestomach squamous-cell carcinomas. These were not significantly elevated compared with the other treatment groups, in which papillomas and squamous-cell carcinomas were not detected. A low incidence of haemangiosarcomas of

the submandibular gland, epididymis and omentum (5%) was also detected in male vinyl carbamate-treated *HRAS2* mice only (Mitsumori *et al*., 1997).

A study was conducted to compare the prevalence of liver neoplasms among five strains of mice. Groups of male mice, 15 days of age, received a single intraperitoneal injection of either 100 µL saline or 100 µL saline that contained vinyl carbamate [stated as pure]. The strains of mice (amount of vinyl carbamate administered and number of mice examined) were B6D2F$_1$ (control, 64 mice; 30 nmol vinyl carbamate, 130 mice), B6C3F$_1$ (control, 138 mice; 30 nmol vinyl carbamate, 70 mice; 150 nmol vinyl carbamate, 128 mice), C3H (control, 73 mice; 30 nmol vinyl carbamate, 181 mice; 150 nmol vinyl carbamate, 139 mice), B6CF$_1$ (control, 97 mice; 30 nmol vinyl carbamate, 114 mice) and C57BL/6 (control, 166 mice; 30 nmol vinyl carbamate, 107 mice; 150 nmol vinyl carbamate, 231 mice). Three to five mice per group were killed at 3–5-week intervals. The first killing of B6C3F$_1$, C57BL/6 and C3H mice was performed at 36 days of age; that of B6D2F$_1$ and B6CF$_1$ mice was performed at 190 days of age. The final killing was conducted when six or fewer mice per group remained; this ranged between 448 and 869 days of age. Overall survival was not indicated. Representative sections from liver masses and lung metastases were examined histologically. The incidence of mice with hepatocellular adenoma, hepatocellular carcinoma and hepatocellular adenoma or carcinoma were: B6D2F$_1$ (control, 6.3%, 7.8% and 14.1%; 30-nmol vinyl carbamate-treated, 37.7%, 38.5% and 59.2%), B6C3F$_1$ (control, 8.0%, 5.1% and 12.3%; 30-nmol vinyl carbamate-treated, 70.0%, 34.3% and 72.9%; 150-nmol vinyl carbamate-treated, 45.3%, 28.1% and 45.3%), C3H (control, 2.7%, 5.5% and 8.2%; 30-nmol vinyl carbamate-treated, 47.5%, 21.5% and 48.6%; 150-nmol vinyl carbamate-treated, 56.1%, 33.8% and 59.7%); B6CF$_1$ (control, 5.2%, 3.1% and 7.2%; 30-nmol vinyl carbamate-treated, 15.8%, 10.5% and 22.8%) and C57BL/6 (control, 1.8%, 0.6% and 2.4%; 30-nmol vinyl carbamate-treated, 34.6%, 18.7% and 43.9%; 150-nmol vinyl carbamate-treated, 43.3%, 22.5% and 46.8%). The incidence of hepatocellular adenoma, hepatocellular carcinoma and hepatocellular adenoma or carcinoma in each of the groups treated with vinyl carbamate was significantly greater than that in the respective control groups [$P \leq 0.03$; one-tailed Fisher's exact test] (Takahashi *et al*., 2002).

Groups of 9–10 male C57BL/6 mice, 6–8 weeks of age, were injected intraperitoneally once or twice with 60 µg/g bw vinyl carbamate [purity not specified] dissolved in saline [volume not specified]. Mice injected once were killed 12 months later; mice that received two injections were dosed at a 1-week interval and killed 6 months after the second injection. No control mice were available. Lung tumours were evaluated histologically. In mice that received a single injection of vinyl carbamate, the incidence of lung adenomas was 5/10 (50%), with a multiplicity (± SE) of 0.50 ± 0.17 tumours/mouse. Lymphoid nodules, which were indistinguishable from epithelial adenomas, were also observed at an incidence of 2/10 (20%) and a multiplicity of 0.20 ± 0.13 tumours/mouse. In mice that received two injections of vinyl carbamate, the incidence of lung adenomas and lymphoid nodules was 1/9 (11%) and 1/9 (11%), with multiplicities of 0.11 ± 0.21 and 0.11 ± 0.21 tumours/mouse, respectively (Miller *et al*., 2003).

(b) Rat

Groups of male and female Fischer rats [initial number not specified], 1 day of age, were given 10 twice-weekly intraperitoneal injections of 92 or 3370 nmol/g bw ethyl carbamate [purity not specified] or five weekly or 10 twice-weekly intraperitoneal injections of 92 nmol/g bw vinyl carbamate (purity not specified but assessed by melting-point, infrared spectroscopy, MS, high-performance liquid chromatography and GC) or 10 twice-weekly intraperitoneal injections of the solvent (10 μL/g bw 0.9% saline). Most of the rats survived the treatment and 17–20 of each sex from each group were weaned. An additional group received five weekly intraperitoneal injections of 380 nmol/g bw vinyl carbamate. Most of these rats died within 3 weeks of being treated, but those remaining were allocated to the experiment. The study was terminated when the rats were 22–23 months old. All animals were subjected to gross necropsy. All tumours were fixed, sectioned and stained with haematoxylin and eosin. The incidence of hepatic carcinomas (mostly mixed hepatocellular-cholangiocellular carcinomas, with a few hepatocellular or cholangiocellular carcinomas) in the male and female rats, respectively, was 0/20 and 0/19 for 10 injections of the solvent, 3/20 (15%) and 0/20 for 10 injections of 92 nmol/g bw ethyl carbamate, 3/18 (17%) and 6/17 (35%) for 10 injections of 3370 nmol/g bw ethyl carbamate, 6/19 (32%) and 4/19 (21%) for five injections of 92 nmol/g bw vinyl carbamate, 6/18 (33%) and 10/20 (50%) for 10 injections of 92 nmol/g bw vinyl carbamate and 8/10 (80%) and 2/3 (67%) for five injections of 380 nmol/g bw vinyl carbamate, and that in all treated groups (males and females combined) was significantly increased compared with the control group, with the exception of rats that received 10 injections of 92 nmol/g bw ethyl carbamate, and that in the group that received 10 injections of 92 nmol/g bw vinyl carbamate was significantly greater than the incidence in the group that received 10 injections of 92 nmol/g bw ethyl carbamate. The incidence of ear duct carcinomas in male and female rats, respectively, was 1/20 (5%) and 0/19 for 10 injections of the solvent, 2/20 (10%) and 0/20 for 10 injections of 92 nmol/g bw ethyl carbamate, 4/18 (22%) and 1/17 (6%) for 10 injections of 3370 nmol/g bw ethyl carbamate, 1/19 (5%) and 2/19 (10%) for five injections of 92 nmol/g bw vinyl carbamate, 4/18 (22%) and 2/20 (10%) for 10 injections of 92 nmol/g bw vinyl carbamate and 4/10 (40%) and 1/3 (33%) for five injections of 380 nmol/g bw vinyl carbamate. The incidence of ear duct carcinomas (males and females combined) was significantly increased in the groups that received 10 injections of 92 nmol/g bw vinyl carbamate and five injections of 380 nmol/g bw vinyl carbamate compared with controls. The incidence of neurofibrosarcomas of the ear lobe in male and female rats, respectively, was 0/20 and 0/19 for 10 injections of the solvent, 0/20 and 0/20 for 10 injections of 92 nmol/g bw ethyl carbamate, 1/18 (5%) and 0/17 for 10 injections of 3370 nmol/g bw ethyl carbamate, 5/19 (26%) and 2/19 (10%) for five injections of 92 nmol/g bw vinyl carbamate, 4/18 (22%) and 1/20 (5%) for 10 injections of 92 nmol/g bw vinyl carbamate and 0/10 and 1/3 for five injections of 380 nmol/g bw vinyl carbamate. The incidence of neurofibrosarcomas of the ear lobe (males and females

combined) was significantly increased in the groups that received five and 10 injections of 92 nmol/g bw vinyl carbamate compared with controls. In addition, the incidence was increased in rats that received 10 injections of 92 nmol/g bw vinyl carbamate compared with rats that received 10 injections of 92 nmol/g bw ethyl carbamate. A low incidence of a variety of other tumours was also observed (Dahl *et al*., 1980).

3.6 References

Allen JW, Stoner GD, Pereira MA *et al.* (1986). Tumorigenesis and genotoxicity of ethyl carbamate and vinyl carbamate in rodent cells. *Cancer Res*, 46: 4911–4915. PMID:3756853

Beland FA, Benson RW, Mellick PW *et al.* (2005). Effect of ethanol on the tumorigenicity of urethane (ethyl carbamate) in B6C3F1 mice. *Food Chem Toxicol*, 43: 1–19. doi:10.1016/j.fct.2004.07.018 PMID:15582191

Berenblum I, Ben-Ishai D, Haran-Ghera N *et al.* (1959). Skin initiating action and lung carcinogenesis by derivatives of urethane (ethyl carbamate) and related compounds. *Biochem Pharmacol*, 2: 168–176. doi:10.1016/0006-2952(59)90065-6 PMID:13799154

Dahl GA, Miller EC, Miller JA (1980). Comparative carcinogenicities and mutagenicities of vinyl carbamate, ethyl carbamate, and ethyl N-hydroxycarbamate. *Cancer Res*, 40: 1194–1203. PMID:7357549

Dahl GA, Miller JA, Miller EC (1978). Vinyl carbamate as a promutagen and a more carcinogenic analog of ethyl carbamate. *Cancer Res*, 38: 3793–3804. PMID:359128

Foley JF, Anderson MW, Stoner GD *et al.* (1991). Proliferative lesions of the mouse lung: progression studies in strain A mice. *Exp Lung Res*, 17: 157–168. doi:10.3109/01902149109064408 PMID:2050022

Ghanayem BI (2007). Inhibition of urethane-induced carcinogenicity in Cyp2e1-/- in comparison to Cyp2e1+/+ mice. *Toxicol Sci*, 95: 331–339. doi:10.1093/toxsci/kfl158 PMID:17093202

IARC. (1974). Some anti-thyroid and related substances, nitrofurans and industrial chemicals. *IARC Monogr Eval Carcinog Risk Chem Man*, 7: 1–326.

Inai K, Arihiro K, Takeshima Y *et al.* (1991). Quantitative risk assessment of carcinogenicity of urethane (ethyl carbamate) on the basis of long-term oral administration to B6C3F1 mice. *Jpn J Cancer Res*, 82: 380–385. PMID:1904417

Iversen OH (1991). Urethan (ethyl carbamate) is an effective promoter of 7,12-dimethylbenz[a]anthracene-induced carcinogenesis in mouse skin two-stage experiments. *Carcinogenesis*, 12: 901–903. doi:10.1093/carcin/12.5.901 PMID:1903092

Kaye AM & Trainin N (1966). Urethan carcinogenesis and nucleic acid metabolism: factors influencing lung adenoma induction. *Cancer Res*, 26: 2206–2212. PMID:5921493

Massey TE, Devereux TR, Maronpot RR *et al.* (1995). High frequency of K-ras mutations in spontaneous and vinyl carbamate-induced lung tumors of relatively resistant B6CF1 (C57BL/6J x BALB/cJ) mice. *Carcinogenesis*, 16: 1065–1069. doi:10.1093/carcin/16.5.1065 PMID:7767966

Miller JA, Cramer JW, Miller EC (1960). The N- and ringhydroxylation of 2-acetylaminofluorene during carcinogenesis in the rat. *Cancer Res*, 20: 950–962. PMID:13853964

Miller YE, Dwyer-Nield LD, Keith RL *et al.* (2003). Induction of a high incidence of lung tumors in C57BL/6 mice with multiple ethyl carbamate injections. *Cancer Lett*, 198: 139–144. doi:10.1016/S0304-3835(03)00309-4 PMID:12957351

Mirvish SS, Smyrk T, Payne S *et al.* (1994). Weak carcinogenicity of 2-hydroxyethyl carbamate in strain A mice: indication that this is not a proximal metabolite of ethyl carbamate. *Cancer Lett*, 77: 1–5. doi:10.1016/0304-3835(94)90340-9 PMID:8162558

Mitsumori K, Wakana S, Yamamoto S *et al.* (1997). Susceptibility of transgenic mice carrying human prototype c-Ha-ras gene in a short-term carcinogenicity study of vinyl carbamate and ras gene analyses of the induced tumors. *Mol Carcinog*, 20: 298–307. doi:10.1002/(SICI)1098-2744(199711)20:3<298::AID-MC6>3.0.CO;2-H PMID:9397190

Mohr U, Dasenbrock C, Tillmann T *et al.* (1999). Possible carcinogenic effects of X-rays in a transgenerational study with CBA mice. *Carcinogenesis*, 20: 325–332. doi:10.1093/carcin/20.2.325 PMID:10069472

National Toxicology Program (2004). *Toxicology and Carinogenesis Studies of Urethane, Ethanol, and Urethane/Ethanol in B6C3F$_1$ Mice (Drinking Water Studies)* (Technical Report Series 510), Research Triangle Park, NC.

Neeper-Bradley TL & Conner MK (1992). Tumor formation and sister chromatid exchange induction by ethyl carbamate: relationships among non-pregnant murine females, gravid dams, and transplacentally exposed offspring. *Teratog Carcinog Mutag*, 12: 167–177. doi:10.1002/tcm.1770120403 PMID:1363158

Nomura T, Hayashi T, Masuyama T *et al.* (1990). Carcinogenicity of sublimed urethane in mice through the respiratory tract. *Jpn J Cancer Res*, 81: 742–746. PMID:2118889

Park K-K, Liem A, Stewart BC, Miller JA (1993). Vinyl carbamate epoxide, a major strong electrophilic, mutagenic and carcinogenic metabolite of vinyl carbamate and ethyl carbamate (urethane). *Carcinogenesis*, 14: 441–450. doi:10.1093/carcin/14.3.441 PMID:8453720

Salmon AG, Zeise L, editors (1991) *Risk of carcinogenesis from urethane exposure.* 1st ed. Boca Raton: CRC Press. 240 p.

Takahashi M, Dinse GE, Foley JF *et al.* (2002). Comparative prevalence, multiplicity, and progression of spontaneous and vinyl carbamate-induced liver lesions in five strains of male mice. *Toxicol Pathol*, 30: 599–605. doi:10.1080/01926230290105776 PMID:12371669

Thorgeirsson UP, Dalgard DW, Reeves J, Adamson RH (1994). Tumor incidence in a chemical carcinogenesis study of nonhuman primates. *Regul Toxicol Pharmacol*, 19: 130–151. doi:10.1006/rtph.1994.1013 PMID:8041912

Wright JA, Marsden AM, Willets JM, Orton TC (1991). Hepatocarcinogenic effect of vinyl carbamate in the C57Bl/10J strain mouse. *Toxicol Pathol*, 19: 258–265. doi:10.1177/019262339101900308 PMID:1664139

Yu W, Sipowicz MA, Haines DC *et al.* (1999). Preconception urethane or chromium(III) treatment of male mice: multiple neoplastic and non-neoplastic changes in offspring. *Toxicol Appl Pharmacol*, 158: 161–176. doi:10.1006/taap.1999.8692 PMID:10406931