IARC MONOGRAPHS

BENZENE

VOLUME 120

This publication represents the views and expert opinions of an IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, which met in Lyon, 10–17 October 2017

LYON, FRANCE - 2018

IARC MONOGRAPHS ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS

International Agency for Research on Cancer

World Health Organization

3. CANCER IN EXPERIMENTAL ANIMALS

Benzene has been evaluated by four *IARC Monographs* Working Groups (IARC, 1974, 1982, 1987, 2012). The Working Group in 2012 concluded that there was *sufficient evidence* in experimental animals for the carcinogenicity of benzene (IARC, 2012).

3.1 Mouse

3.1.1 Inhalation

See Table 3.1

Numerous studies have been conducted in mice exposed to benzene by inhalation. Even though some of these studies focused on the effects of benzene in transgenic mice, data from these studies reporting the effects of benzene in wildtype mice are included in this section.

C57BL/6 female mice (age, 7–9 weeks) were exposed to clean air (116 mice) or benzene at 300 ppm [purity not reported] (118 mice) for 6 hours per day, 5 days per week, for 16 weeks in whole-body inhalation chambers. The 116 clean-air controls and 118 mice exposed to benzene were reduced to 88 and 90 mice, respectively, by interim killing for assays for haematopoietic stem cells. Preliminary observations were reported 64 weeks after beginning exposure (Cronkite et al., 1984). During this period there was increased mortality in the mice exposed to benzene: one of the clean-air control mice died and 10 mice exposed to benzene died. The dead control mouse did not have lymphoma or leukaemia. Of the 10 mice exposed to benzene that died, 6 had lymphoma of the thymus gland, 2 had unspecified lymphomas, 1 did not have lymphoma or leukaemia, and 1 carcass was lost due to cannibalism and autolysis of tissues. The incidence of lymphoma or leukaemia 64 weeks after beginning exposure was 8/89 (9.0%) in mice exposed to benzene compared with 0/88 in control mice. [These tumour incidences indicated a significant increase in the incidence of lymphoma or leukaemia [$P = 0.007$, Fisher exact test]; however, because 87 of 88 clean-air control mice and 80 of 89 mice exposed to benzene were not comprehensively examined, the actual tumour incidences in the control groups and groups exposed to benzene were unknown. The study was considered inadequate for the evaluation.]

C57BL/6 female mice (age, 8–12 weeks) were exposed to clean air (88 mice) or benzene at a dose of 300 ppm [purity not reported] (89 mice) for 6 hours per day, 5 days per week, for 16 weeks in inhalation chambers, and then observed for their lifetimes. The observations reported were made 692 days after beginning exposure (580 days after the end of the exposure period) (Cronkite et al., 1985). During this period, there was increased mortality in the mice exposed to benzene: 23 deaths in the control group and 48 deaths in the group exposed to benzene were observed. There was a significantly higher incidence of tumours in the mice exposed to benzene that had died by day 692 of the experiment: leukaemia (all

Table 3.1 Studies of carcinogenicity in mice exposed to benzene by inhalation

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Mouse, C57BL/6 (F) 7–9 wk 64 wk Cronkite et al. (1984)	Inhalation (whole-body exposure) Benzene, reagent grade Air 0, 300 ppm (in air) 6 h/d, 5 d/wk, 16 wk 88, 90 87, 80	*Haematopoietic and lymphoid tissues*: lymphoma/leukaemia 0/88, 8/89	See comments	Principal limitations: preliminary data obtained from dead or moribund animals; animals alive at 64 wk were not assessed Surviving mice were not examined, so the tumour incidence in the control and benzene-exposed populations is unknown; the study was considered inadequate
Full carcinogenicity Mouse, C57BL/6 (F) 8–12 wk 692 d Cronkite et al. (1985)	Inhalation (whole-body exposure) Benzene, probably reagent grade Air 0, 300 ppm (in air) 6 h/d, 5 d/wk, 16 wk 88, 89 65, 41	*Haematopoietic and lymphoid tissues* Thymic lymphoma 1/88, 10/89 Non-thymic lymphoma 2/88, 6/89 Myelogenous leukaemia 3/88, 0/89 Unspecified leukaemia 2/88, 4/89 Leukaemia (all types) 8/88, 20/89 *Zymbal gland*: benign and malignant epidermoid tumours and lymphoepithelioma 1/88, 16/89 *Ovary*: unspecified solid tumours 0/88, 8/89 *Liver*: hepatoma (not specified) 1/88, 1/89 *Other neoplasms*: unspecified solid tumours 2/88, 4/89	See comments See comments See comments See comments See comments See comments See comments See comments See comments	Principal limitations: data obtained from dead or moribund animals; animals alive at 692 d after the commencement of the study were not assessed; the data presented in Table 1 appear to be the continuing observations of the study reported in Cronkite et al. (1984), although this is not specifically stated Surviving mice were not examined, so tumour incidence in the control and in the benzene-exposed populations was therefore unknown; the study was considered inadequate

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Mouse, CBA/Ca BNL (M) 12 wk Lifetime Cronkite et al. (1989)	Inhalation (whole-body exposure) Benzene, purity NR Air 0, 300 ppm (in air) 6 h/d, 5 d/wk, 16 wk 60, 57 NR	*Haematopoietic and lymphoid tissues* Lymphomatous neoplasms 7/60, 1/57 Myelogenous neoplasms 0/60, 11/57* *Liver*: hepatoma (not specified) 16/60, 6/57 *Other neoplasms*: unspecified solid tumours 13/60, 30/57*	 NS *$P < 0.001$ NS *$P < 0.001$	Principal strengths: lifetime study; studies in male and female mice
Full carcinogenicity Mouse, CBA/Ca BNL (F) 12 wk Lifetime Cronkite et al. (1989)	Inhalation (whole-body exposure) Benzene, purity NR Air 0, 300 ppm (in air) 6 h/d, 5 d/wk, 16 wk 60, 54 NR	*Haematopoietic and lymphoid tissues* Lymphomatous neoplasms 5/60, 4/54 Myelogenous neoplasms 1/60, 6/54* *Liver*: hepatoma (not specified) 8/60, 0/54 *Other neoplasms*: unspecified solid tumours 21/60, 43/54*	 NS *$P = 0.040$ (chi-squared test) NS (for an increase) *$P < 0.001$	Principal strengths: lifetime study; studies in male and female mice *P* values for the difference in the incidence of myelogenous neoplasms between control and benzene-exposed females are different when using the one-tail and the two-tail Fisher exact test (as calculated by the Working Group): 0.0420 and 0.0514 (not significant), respectively
Full carcinogenicity Mouse, CBA/Ca BNL (M) 12 wk Lifetime Cronkite et al. (1989)	Inhalation (whole-body exposure) Benzene, purity NR Air 0, 100 ppm (in air) 6 h/d, 5 d/wk, 16 wk 70, 85 NR	*Haematopoietic and lymphoid tissues* Lymphomatous neoplasms 12/70, 7/85 Myelogenous neoplasms 0/70, 2/85 *Liver*: hepatoma (not specified) 27/70, 35/85 *Other neoplasms*: unspecified solid tumours 14/70, 38/85*	 NS NS NS *$P = 0.001$	Principal strengths: lifetime study

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Mouse, CBA/Ca (M) 10–12 wk 22 mo Farris et al. (1993)	Inhalation (whole-body exposure) Benzene, purity NR but certified HPLC grade Air 0, 300 ppm (in air) 6 h/d, 5 d/wk, 16 wk 125, 125 75, 25	*Haematopoietic and lymphoid tissues*: malignant lymphoma 2/119, 14/118* *Preputial gland*: squamous cell carcinoma 0/118, 71/118* *Lung*: adenoma 17/119, 42/118* *Zymbal gland*: carcinoma 1/125, 14/125 *Forestomach*: squamous cell carcinoma 0/125, 9/125 *Harderian gland*: adenoma 6/125, 7/125	*$P < 0.01$ (table), $P < 0.002$ (text) *$P < 0.01$ *$P < 0.01$ [$P < 0.01$]; see comments [$P < 0.01$]; see comments NS; see comments	Principal strengths: covers most of the lifespan Benzene decreased survival ($P < 0.01$); the incidence of Zymbal gland carcinoma and of forestomach squamous cell carcinoma was significantly increased by Fisher's exact test: $P < 0.01$ and $P < 0.01$, respectively; Zymbal gland, forestomach, and Harderian gland were examined microscopically only when gross lesions were evident
Full carcinogenicity Mouse, AKR (NR) NR Lifetime Goldstein et al. (1982)	Inhalation Benzene, purity NR Air 0, 100 ppm (in air) 6 h/d, 5 d/wk 50, 50 NR	*Haematopoietic and lymphoid tissues*: myelogenous leukaemia 0/50, 0/50	NS	Principal strengths: lifetime study Principal limitations: only myeloproliferative disorders were assessed; methods were poorly described
Full carcinogenicity Mouse, AKR (NR) NR Lifetime Goldstein et al. (1982)	Inhalation Benzene, purity NR Air 0, 300 ppm (in air) 6 h/d, 5 d/wk 80, 80 NR	*Haematopoietic and lymphoid tissues*: myelogenous leukaemia 0/80, 0/80	NS	Principal strengths: lifetime study Principal limitations: only myeloproliferative disorders were assessed; methods were poorly described

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Mouse, C57Bl (NR) NR Lifetime Goldstein et al. (1982)	Inhalation Benzene, purity NR Air 0, 300 ppm (in air) 6 h/d, 5 d/wk 40, 40 NR	*Haematopoietic and lymphoid tissues*: myelogenous leukaemia 0/40, 0/40	NS	Principal strengths: lifetime study Principal limitations: only myeloproliferative disorders were assessed; methods were poorly described
Full carcinogenicity Mouse, CD-1 (NR) NR Lifetime Goldstein et al. (1982)	Inhalation Benzene, purity NR Air 0, 300 ppm (in air) 6 h/d, 5 d/wk 40, 40 NR	*Haematopoietic and lymphoid tissues*: myelogenous leukaemia 0/40, 2/40	NS; see comments	Principal strengths: lifetime study Principal limitations: only myeloproliferative disorders were assessed; methods were poorly described Spontaneous cases of myelogenous leukaemia are rare in CD-1 mice; the authors argue that the two occurrences of myelogenous leukaemia are likely due to benzene inhalation
Full carcinogenicity Mouse, C57BL/6 (wildtype) (M) 8 wk Lifetime Kawasaki et al. (2009)	Inhalation (whole-body exposure) Benzene, purity NR (purchased from Wako Fine Chemicals, Japan) Air 0, 33, 100, 300 ppm (in air) 6 h/d, 5 d/wk, 26 wk 20, 19, 19, 18 NR	*Haematopoietic and lymphoid tissues* Thymic lymphoma 0/20, 0/19, 2/19, 5/18* Non-thymic lymphoma 2/20, 4/19, 1/19, 5/18 Myeloid leukaemia 0/20, 0/19, 0/19, 0/18 Neoplasms of haematopoietic and lymphoid tissues (combined) 2/20, 4/19, 3/19, 10/18* *Other neoplasms*: solid tumours (not specified) excluding lymphomas 3/20, 3/19, 8/19, 2/18	 *$P < 0.05$ [$P = 0.004$] (Cochran–Armitage trend test) NS NS *$P < 0.05$ NS	Principal strengths: lifetime study

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Mouse, C3H/He (wildtype) (M) 8 wk Lifetime Kawasaki et al. (2009)	Inhalation (whole-body exposure) Benzene, purity NR (purchased from Wako Fine Chemicals, Japan) Air 0, 100, 300 ppm (in air) 6 h/d, 5 d/wk, 26 wk 23, 24, 23 NR	*Haematopoietic and lymphoid tissues* Thymic lymphoma 0/23, 4/24, 0/23 Non-thymic lymphoma 2/23, 2/24, 5/23 Myeloid leukaemia 0/23, 0/24, 2/23 Neoplasms of haematopoietic and lymphoid tissues (combined) 2/23, 6/24, 7/23 *Other neoplasms*: solid tumours (not specified) excluding lymphomas 11/23, 5/24, 8/23	 [$P = 0.0031$] (Cochran–Armitage trend test) NS NS NS NS	Principal strengths: lifetime study
Full carcinogenicity Mouse, C57BL/6 (wildtype) (NR) NR Lifetime Li et al. (2006)	Inhalation (whole-body exposure) Benzene, purity NR (purchased from Wako Fine Chemicals, Japan) Air 0, 300 ppm 6 h/d, 5 d/wk, 26 wk 8, 10 NR	*Haematopoietic and lymphoid tissues* Thymic lymphoma 0/8, 3/10 Non-thymic lymphoma 6/8, 5/10	 [NS] [NS]; see comments	Principal strengths: lifetime study Principal limitations: small numbers of animals were used The time to non-thymic lymphoma was shorter in benzene-exposed mice compared with controls
Full carcinogenicity Mouse, AKR/J (M) 8 wk Lifetime Snyder et al. (1980)	Inhalation (whole-body exposure) Benzene, purity NR Air 0, 100 ppm (in air) 6 h/d, 5 d/wk 50, 50 NR	*Haematopoietic and lymphoid tissues*: malignant lymphoma 24/50, 29/49	 NS	Principal strengths: lifetime study

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Mouse, C57BL/6J (M) 8 wk Lifetime Snyder et al. (1980)	Inhalation (whole-body exposure) Benzene, purity NR Air 0, 300 ppm (in air) 6 h/d, 5 d/wk 40, 40 NR	*Haematopoietic and lymphoid tissues* Haematopoietic neoplasms (combined) 2/40, 8/40* Bone marrow hyperplasia in animals without haematopoietic neoplasm 0/38, 13/32*	 *$P < 0.005$; see comments *$P < 0.001$	Principal strengths: lifetime study Principal limitations: poor data presentation Using the two-tail Fisher exact test to compare the incidence of haematopoietic neoplasms in clean air and benzene-exposed C57BL/6J mice, the Working Group determined that the *P* value was NS (0.0872). On the other hand, using the log-rank (χ^2) test, which compares events and time to event, the difference in malignant lymphoma incidence between control and benzene-exposed mice was found to be significant by the authors ($P < 0.005$). The significance found by the authors therefore depends on tumour induction time
Full carcinogenicity Mouse, CD-1 (M) 8 wk Lifetime Snyder et al. (1988)	Inhalation (whole-body exposure) Benzene, purity NR Air 0, 1200 ppm (in air) 6 h/d, 5 d/wk, 10 wk 80, 80 71 (at risk), 71 (at risk)	*Haematopoietic and lymphoid tissues*: leukaemia/lymphoma 11/71, 11/71 *Zymbal gland*: carcinoma 0/71, 4/71* *Lung*: adenoma 17/71, 33/71* *All sites* Malignant tumours 22/71, 24/71* Benign tumours 21/71, 35/71* Benign and malignant tumours 36/71, 45/71*	 NS *$P < 0.05$ [NS]; see comments *$P < 0.001$ *$P < 0.05$ [NS]; see comments *$P < 0.001$ *$P < 0.001$ [NS]; see comments	Principal strengths: lifetime study Principal limitations: short duration of exposure; use of the log-rank (χ^2) test is questionable Using the two-tail Fisher exact test, the Working Group determined that only the incidence of lung adenoma and of benign tumours was significantly increased in benzene-exposed mice ($P = 0.0081$ and 0.0252, respectively)

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Mouse, C57BL/6J (M) 8 wk Lifetime Snyder et al. (1988)	Inhalation (whole-body exposure) Benzene, purity NR Air 0, 1200 ppm (in air) 6 h/d, 5 d/wk, 10 wk 80, 80 67 (at risk), 68 (at risk)	*Haematopoietic and lymphoid tissues*: leukaemia/lymphoma 15/67, 11/68 *Zymbal gland*: carcinoma 0/67, 4/68 *Lung*: adenoma 11/67, 15/68 *All sites* Malignant tumours 19/67, 15/68 Benign tumours 13/67, 16/68 Benign and malignant tumours 28/67, 30/68	 NS NS NS NS NS NS	Principal strengths: lifetime study Principal limitation: short duration of exposure
Full carcinogenicity Mouse, CD-1 (M) 8 wk Lifetime Snyder et al. (1988)	Inhalation (whole-body exposure) Benzene, purity NR Air 0, 300 ppm (in air) 6 h/d, 5 d/wk, 1 wk then 2 wk unexposed, for life 60, 60 46 (at risk), 54 (at risk)	*Haematopoietic and lymphoid tissues*: leukaemia/lymphoma 1/46, 7/54* *Zymbal gland*: carcinoma 0/46, 2/54 *Lung*: adenoma 3/46, 14/54* *All sites* Malignant tumours 1/46, 12/54* Benign tumours 3/46, 15/54* Benign and malignant tumours 4/46, 25/54*	 *[$P < 0.05$] (one-tail Fisher exact test) NS *$P < 0.005$ *$P < 0.005$ *$P < 0.001$ *$P < 0.001$	Principal strengths: lifetime study In the group of exposed mice, the Working Group determined that increases in the incidence of lung adenoma, malignant tumours, benign tumours, and of total tumours were also significant by the two-tail Fisher exact test, with *P* values of 0.015, 0.003, 0.008, and < 0.0001, respectively

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Mouse, C57BL/6J (M) 8 wk Lifetime Snyder et al. (1988)	Inhalation (whole-body exposure) Benzene, purity NR Air 0, 300 ppm (in air) 6 h/d, 5 d/wk, 1 wk then 2 wk unexposed, for life 60, 60 46 (at risk), 54 (at risk)	*Haematopoietic and lymphoid tissues*: leukaemia/lymphoma 1/46, 3/54 *Zymbal gland*: carcinoma 0/46, 19/54* *Lung*: adenoma 5/46, 3/54 *All sites* Malignant tumours 2/46, 24/54* Benign tumours 6/46, 5/54 Benign and malignant tumours 8/46, 25/54*	 NS *$P < 0.001$ NS *$P < 0.001$ NS *$P < 0.001$	Principal strengths: lifetime study In the group of exposed mice, the Working Group determined that increases in the incidence of Zymbal gland carcinoma, malignant tumours, and of total tumours were also significant by the two-tail Fisher exact test, with P values of < 0.0001, < 0.0001, and 0.003, respectively

d, day(s); F, female; h, hour(s); HPLC, high-performance liquid chromatography; M, male; mo, month(s); NR, not reported; NS, not significant; ppm, parts per million; wk, week(s)

types): control, 8/88 (9.1%) and benzene, 20/89 (22.5%) [$P = 0.0223$, Fisher]; tumours of the Zymbal gland: control, 1/88 (1.1%) and benzene, 16/89 (18.0%) [$P = 0.001$, Fisher]; tumours of the ovary: control, 0/88 and benzene, 8/89 (9.0%) [$P = 0.0066$, Fisher]; hepatoma [not further specified]: control, 1/88 (1.1%) and benzene, 1/89 (1.1%); and other tumours: control, 2/88 (2.3%) and benzene, 4/89 (4.5%). [These incidences indicated a significant increase in carcinogenicity in mice exposed to benzene; however, the animals that were still alive at experimental day 692 (65 control and 41 exposed to benzene) were not comprehensively examined. The actual tumour incidences in the control group and the group exposed to benzene were therefore unknown. The similarities between the mice described in Table 1 of the 1985 article and Table 1 of the 1984 article authored by Cronkite et al. suggested that these were the same groups of experimental animals; this is not specifically stated in the 1985 article, however (Cronkite et al., 1984, 1985). The study was considered inadequate for the evaluation.]

Six groups of CBA/Ca BNL male or female mice (age, 12 weeks) were exposed to clean air or benzene at a dose of 100 or 300 ppm [purity not reported] for 6 hours per day, 5 days per week, for 16 weeks in inhalation chambers and then observed for their lifetimes (Cronkite et al., 1989). Groups 1 and 2 consisted of 60 males exposed to clean air and 57 males exposed to benzene at 300 ppm; groups 3 and 4 consisted of 60 females exposed to clean air and 54 females exposed to benzene at 300 ppm; and groups 5 and 6 consisted of 70 males exposed to clean air and 85 males exposed to benzene at 100 ppm. Mice exposed to benzene at 300 pm, but not 100 ppm, had shorter lifespans than controls. Median lifespans of males and females were 1030 and 1100 days (clean air) and 510 and 580 days (300 ppm benzene), respectively, while the median lifespans of males exposed to clean air and 100 ppm benzene were 1020 and 1000 days, respectively. Neither lymphomatous neoplasms nor hepatoma were increased in male or female mice exposed to benzene at 300 ppm; however, the mice exposed to benzene at 300 ppm had increased incidences of myelogenous neoplasms (controls, 0/60 (males) and 1/60 (females); benzene, 11/57 (males) ($P < 0.001$) and 6/54 (females) ($P = 0.040$) [$P = 0.0420$, one-tail Fisher exact test; $P = 0.0514$ (not significant), two-tail Fisher exact test]) and solid tumours other than hepatoma or of the haematopoietic or lymphoid tissues (controls, 13/60 (males) and 21/60 (females); benzene, 30/57 (males) ($P < 0.001$) and 43/54 (females) ($P < 0.001$)). Male mice exposed to benzene at 100 ppm also had an increased incidence of solid tumours other than hepatoma or of the haematopoietic or lymphoid tissues (controls, 14/70; benzene, 38/85 ($P = 0.001$)).

Groups of 125 CBA/Ca male mice (age, 10–12 weeks) were exposed to clean air or benzene [high-performance liquid chromatography grade] at a dose of 300 ppm for 6 hours per day, 5 days per week, for 16 weeks in inhalation chambers, and then observed for up to 18 months (Farris et al., 1993). There was a significant decrease in survival in the group exposed to benzene ($P < 0.01$). There was a significant increase in the incidences of malignant lymphoma (control, 2/119; benzene, 14/118; $P < 0.01$), squamous cell carcinoma of the preputial gland (control, 0/118; benzene, 71/118; $P < 0.01$), adenoma of the lung (control, 17/119; benzene, 42/118; $P < 0.01$), carcinoma of the Zymbal gland (control, 1/125; benzene, 14/125 [$P < 0.01$, Fisher]), and squamous cell carcinoma of the forestomach (control, 0/125; benzene, 9/125 [$P < 0.01$, Fisher]).

Groups of 50–80 AKR mice, 40 C57Bl mice, and 40 CD-1 mice [sex and age not reported] were exposed to clean air or benzene at a dose of 100 or 300 ppm [purity not reported] in inhalation chambers for 6 hours per day, 5 days per week, for life (Goldstein et al., 1982). Of the CD-1 mice exposed to benzene at 300 ppm, one developed chronic myelogenous leukaemia

and another developed acute myelogenous leukaemia. [The Working Group noted that only myeloproliferative disorders were assessed and that, while there was no statistical increase in the incidence of myelogenous leukaemia in mice exposed to benzene compared with controls, spontaneous cases of myelogenous leukaemia are rare in CD-1 mice. The authors argued that "the absence of a background incidence of acute and chronic myelogenous leukaemia in CD-1 mice … suggests that the present observations are due to a direct effect of benzene inhalation."] No leukaemias were observed in AKR or C57Bl mice. [The Working Group also noted the very low incidence of neoplasms of the haematopoietic and lymphoid tissues in all three strains of mice tested.]

In lifetime studies, groups of male C57BL/6 wildtype (18–20 animals per group), heterozygous *Trp53*-deficient or homozygous Trp53-deficient mice (age, 8 weeks) were exposed to clean air or benzene at a dose of 33, 100, or 300 ppm [purity not reported, chemical grade] for 6 hours per day, 5 days per week, for 26 weeks in inhalation chambers, and groups of 18–24 male C3H/He wildtype (23–24 animals per group), heterozygous *Trp53*-deficient or homozygous Trp53-deficient mice (age, 8 weeks) were exposed to clean air or benzene at a dose of 100 or 300 ppm for 6 hours per day, 5 days per week, for 26 weeks in inhalation chambers (Kawasaki et al., 2009; see also Section 3.3.1). Wildtype mice of both strains exposed to benzene at 300 ppm had decreased survival rates ($P < 10^{-5}$). Wildtype C57BL/6 mice exposed to benzene at 300 ppm had significant increases in the incidences of lymphoma of the thymus gland (control, 0/20; benzene, 5/18; $P < 0.05$) and total neoplasms of the haematopoietic and lymphoid tissues (control, 2/20; benzene, 10/18; $P < 0.05$). Wildtype C3H/He mice exposed to benzene at 300 ppm had a non-significant increase in the incidence of total neoplasms of the haematopoietic and lymphoid tissues (control, 2/23; benzene, 7/23; not significant), and two of these mice developed myeloid leukaemia (compared with none from the control group). There was also a significant positive trend in the incidence of lymphoma of the thymus gland for both strains of mice. There was no increase in the incidence of solid tumours in any of the groups of wildtype mice exposed to benzene.

In a study using C57BL/6 h-Trx-Tg transgenic mice, Li et al. (2006) exposed 8 wildtype C57BL/6 mice to clean air and 10 wildtype C57BL/6 mice [sex not reported] to benzene at 300 ppm [purity not reported, chemical grade] in inhalation chambers for 6 hours per day, 5 days per week, for 26 weeks, and monitored the mice for their lifetimes. Until the mice reached an age of approximately 2 years, the proportion surviving in wildtype mice exposed to benzene was lower than in wildtype mice exposed to clean air. The cumulative incidence of thymic lymphoma in clean-air controls and wildtype mice exposed to benzene was 0/8 and 3/10 [no significant increase], respectively, and the cumulative incidence of non-thymic lymphoma was 6/8 and 5/10, respectively; however, the time to non-thymic lymphoma was shorter in mice exposed to benzene compared with controls.

Groups of 50 male AKR/J mice and 40 male C57BL/6J mice (age, 8 weeks) were exposed to clean air or benzene at a dose of 100 ppm [purity not reported] (AKR mice) or 300 ppm (C57BL/6J mice) in inhalation chambers for 6 hours per day, 5 days per week, for life (Snyder et al., 1980). In the AKR mice, there was no difference in weight gain or median survival between those exposed to clean air and those exposed to benzene at 100 ppm. In contrast, C57BL/6J mice exposed to benzene at 300 ppm had a decreased weight gain and median survival: median survival of mice exposed to benzene was 41 weeks compared with 75 weeks for the controls. Inhalation of benzene at 100 ppm did not increase the incidence of malignant lymphoma in AKR mice: malignant lymphoma was found in 24/50 mice exposed to clean air and 29/49 mice exposed to benzene.

Haematopoietic neoplasms were found in 2/40 C57BL/6J mice exposed to clean air and 8/40 C57BL/6J mice exposed to benzene at 300 ppm ($P < 0.005$, log-rank (χ^2) test [$P = 0.0872$, two-tail Fisher exact test]), and hyperplasia of the bone marrow without neoplasia was found in 0/38 control mice and 13/32 mice exposed to benzene ($P < 0.001$, log-rank (χ^2) test [$P < 0.001$, Fisher exact test]). [Using the two-tail Fisher exact test, the incidence of haematopoietic neoplasms was not statistically significantly increased in C57BL/6J mice exposed to benzene at 300 ppm. On the other hand, using the log-rank test, which compares events and times to event, the difference in haematopoietic neoplasm incidence between control groups and mice exposed to benzene was found to be significant. The significance found by Snyder et al. (1980) therefore depends on tumour induction time. In support of the authors' conclusion that C57BL/6J mice exposed to benzene at 300 ppm had a significant increase in the incidence of haematopoietic neoplasms, hyperplasia of the bone marrow without neoplasia was significantly increased in mice exposed to benzene.]

Male CD-1 and C57BL/6J mice (age, 8 weeks) were exposed to clean air or benzene at a dose of 300 or 1200 ppm [purity not reported] in inhalation chambers (Snyder et al., 1988). In a first protocol, groups of 80 CD-1 and 80 C57BL/6 mice were exposed to clean air or to benzene at 1200 ppm for 6 hours per day, 5 days per week, for 10 weeks, and then observed for their lifetimes. In a second protocol, groups of 60 CD-1 and 60 C57BL/6 mice were exposed to clean air or to benzene at 300 ppm for 6 hours per day, 5 days per week, for 1 week, followed by non-exposure for 2 weeks; this regimen was repeated for life. Exposure to benzene did not affect the mortality rate of either CD-1 or C57BL/6 mice; however, for clean-air controls, the 50% mortality rate occurred earlier in mice exposed according to the first protocol than in mice exposed according to the second protocol (approximately 460 days vs 600 days for CD-1 mice, and 740 days vs 840 days for C57BL/6 mice). Tumour incidences in CD-1 mice exposed to clean air or benzene at 1200 ppm were: malignant tumours, 22/71 or 24/71; benign tumours, 21/71 or 35/71; total tumours, 36/71 or 45/71; adenoma of the lung, 17/71 or 33/71; leukaemia/lymphoma, 11/71 or 11/71; and carcinoma of the Zymbal gland, 0/71 or 4/71, respectively. [Using the log-rank (χ^2) test, Snyder et al. (1988) reported that the increases in the incidence of malignant tumours, benign tumours, total tumours, adenoma of the lung, and carcinoma of the Zymbal gland were significant; however, using the two-tail Fisher exact test, the Working Group determined that only the incidence of benign tumours and of adenoma of the lung was significantly increased in the exposed mice.] Tumour incidence was not increased in C57BL/6 mice exposed to benzene at 1200 ppm. Tumour incidence in CD-1 mice intermittently exposed to clean air or benzene at 300 ppm was: malignant tumours, 1/46 or 12/54; benign tumours, 3/46 or 15/54; total tumours, 4/46 or 25/54; adenoma of the lung, 3/46 or 14/54; leukaemia/lymphoma, 1/46 or 7/54 [$P < 0.05$, one-tail Fisher exact test]; and carcinoma of the Zymbal gland, 0/46 or 2/54. [Using the two-tail Fisher exact test, the Working Group confirmed the conclusions of Snyder et al. that the incidence of total tumours, malignant tumours, benign tumours, and of adenoma of the lung was significantly increased in mice exposed to benzene.] Tumour incidence in C57BL/6 mice intermittently exposed to clean air or benzene at 300 ppm was: malignant tumours, 2/46 or 24/54; benign tumours, 6/46 or 5/54; total tumours, 8/46 or 25/54; adenoma of the lung, 5/46 or 3/54; leukaemia/lymphoma, 1/46 or 3/54; and carcinoma of the Zymbal gland, 0/46 or 19/54. [Using the two-tail Fisher exact test, the Working Group confirmed the conclusions of Snyder et al. that the incidence of total tumours, malignant tumours, and of carcinoma of the Zymbal gland was significantly increased in mice exposed to benzene. The Working Group noted the short duration of exposure in the first protocol.]

3.1.2 Oral administration

See Table 3.2

Good laboratory practice (GLP) studies of carcinogenicity with benzene (purity, > 99.7%) were conducted in groups of 50 B6C3F$_1$ mice of each sex (age, 6.5–8.5 weeks); four groups were given benzene at a dose of 0 (control), 25, 50, or 100 mg/kg body weight (bw) in corn oil by gavage 5 days per week, for 103 weeks (NTP, 1986; Huff et al., 1989). At the age of 2 years, mean body weights of male and female mice given the high dose were significantly decreased. Survival of male and female mice given the high dose was also significantly decreased (males, 28/50 (control), 23/50, 18/50, 7/50; females, 30/50 (control), 26/50, 24/50, 18/50). Most mice exposed to benzene that died before week 103 had neoplasia. Compound-related non-neoplastic and neoplastic effects were found for the adrenal gland, forestomach, Harderian gland, haematopoietic and lymphoid tissues, liver, lung, mammary gland, ovary, preputial gland, and Zymbal gland.

In males and females, benzene caused a significant increase in the incidence of the following lesions, with a significant positive trend: epithelial hyperplasia and carcinoma of the Zymbal gland; hyperplasia of the bone marrow (haematopoietic system), and lymphoma or leukaemia (combined); alveolar epithelial hyperplasia, bronchioloalveolar adenoma and carcinoma, and bronchioloalveolar adenoma or carcinoma (combined); hyperplasia and adenoma or carcinoma (combined) of the Harderian gland; epithelial hyperplasia, hyperkeratosis, and squamous cell papilloma of the forestomach; and hyperplasia of the adrenal gland. In males only, benzene caused a significant increase in the incidence of adenoma of the Harderian gland and pheochromocytoma of the adrenal gland. In females only, benzene caused a significant increase in the incidence of carcinoma of the Harderian gland, with a significant positive trend.

In male mice, benzene caused significant increases in the incidence, and a significant positive trend in the incidence, of hyperplasia of the preputial gland, squamous cell carcinoma of the preputial gland, carcinoma (not otherwise specified) of the preputial gland, and of carcinoma (all types) of the preputial gland. There was also a small but significant increase in the incidence of hepatocellular carcinoma and of hepatocellular adenoma or carcinoma (combined).

In female mice, benzene caused significant increases in the incidence, and a significant positive trend in the incidence, of tubular adenoma of the ovary, granulosa cell tumours [benign] of the ovary, granulosa cell tumours or carcinoma (combined) of the ovary, mixed tumours (benign) of the ovary, carcinoma of the mammary gland, and of carcinosarcoma of the mammary gland, and a significant increase in the incidence of hepatocellular adenoma and hepatocellular adenoma or carcinoma (combined) (NTP, 1986; Huff et al., 1989). [The Working Group noted that this was a well-conducted GLP study with multiple doses, using both males and females, covering most of the lifespan, and with complete histopathology. Mice treated with benzene had lower body weights and survival.]

A group of 40 male and 40 female Swiss mice (age, 7 weeks) was given benzene (purity, > 99.93%) at a dose of 0 (control) or 500 mg/kg bw in olive oil by stomach tube once per day, 4–5 days per week, for 78 weeks. Mice were observed for life. The authors stated that body weights were lower in treated mice, and particularly in males. Survival was comparable among groups. Necropsies were performed on all animals, with histopathological examinations on 29 tissues and organs (several with multiple sections, e.g. gastrointestinal tract), and all lesions. There were significant increases in the incidence of primary tumours (benign and malignant) (male, 15/40 vs 24/40; female, 16/40 vs 32/40), malignant tumours (female, 11/40 vs 28/40), total tumours [mainly adenomas] of the lung (male, 3/40 vs 17/40; female, 4/40 vs 15/40)

Table 3.2 Studies of carcinogenicity in mice exposed to benzene by gavage or intraperitoneal injection

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence and/or multiplicity of tumours	Significance	Comments
Full carcinogenicity Mouse, B6C3F$_1$ (M) 6.5–8.5 wk 103 wk NTP (1986)	Gavage Benzene, > 99.7% Corn oil 0, 25, 50, 100 mg/kg bw 5 days/wk, 103 wk 50, 50, 50, 50 28, 23, 18, 7	*Zymbal gland*: squamous cell carcinoma 0/43*, 1/34, 4/40**, 21/39***	*P < 0.001 (trend), **P = 0.012, ***P < 0.001 (life-table test)	Principal strengths: 2 yr bioassay; control and three dose groups; complete histopathology; well-conducted GLP study; studies in both male and female mice Principal limitations: much of the lowered body weights and slightly reduced survival could be attributed to tumour-bearing animals; the survival of high-dose groups of male and female mice was significantly lower than respective vehicle control groups; mean body weights of high-dose groups of male and female mice were lower than vehicle controls The incidence of preneoplastic hyperplasia was increased in tumours of Zymbal gland, preputial gland, Harderian gland, lung, haematopoietic system (bone marrow), forestomach, and adrenal gland caused by benzene
		Haematopoietic and lymphoid tissues Lymphoma 4/49*, 9/48, 9/50**, 15/49***	*P < 0.001 (trend), **P = 0.030, ***P < 0.001 (life-table test)	
		Lymphoma or leukaemia (combined) 4/49*, 10/48**, 10/50***, 15/49****	*P < 0.001 (trend), **P = 0.048, ***P = 0.018, ****P < 0.001 (life-table test) Leukaemia in one low-dose and one intermediate-dose group mouse	
		Lung Alveolar/bronchiolar adenoma 6/49*, 6/48, 8/50, 12/49**	*P < 0.001 (trend), **P = 0.005 (life-table test)	
		Alveolar/bronchiolar carcinoma 5/49*, 11/48, 12/50**, 14/49***	*P < 0.001 (trend), **P = 0.017, ***P < 0.001 (life-table test)	
		Alveolar/bronchiolar adenoma or carcinoma (combined) 10/49*, 16/48, 19/50**, 21/49***	*P < 0.001 (trend), **P = 0.007, ***P < 0.001 (life-table test)	
		Adrenal gland: pheochromocytoma 1/47, 1/48, 7/49*, 1/46	*P = 0.010 (life-table test)	
		Preputial gland Squamous cell carcinoma 0/21*, 3/28, 18/29**, 28/35**	*P < 0.001 (trend), **P < 0.001 (life-table test)	

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence and/or multiplicity of tumours	Significance	Comments
Full carcinogenicity Mouse, B6C3F$_1$ (M) 6.5–8.5 wk 103 wk NTP (1986) (cont.)		Carcinoma, NOS 0/21*, 2/28, 1/29, 3/35**	*P < 0.019 (trend), **P < 0.043 (life-table test)	
		Carcinoma (all types) 0/21*, 5/28, 19/29**, 31/35**	*P < 0.001 (trend), **P < 0.001 (life-table test)	
		Harderian gland Adenoma 0/49*, 9/46**, 13/49***, 11/48***	*P < 0.001 (trend), **P = 0.001, ***P < 0.001 (life-table test)	
		Adenoma or carcinoma (combined) 1/49*, 10/46**, 13/49***, 14/48***	*P < 0.001 (trend), **P = 0.002, ***P < 0.001 (life-table test)	
		Forestomach Squamous cell papilloma 2/45*, 1/42, 2/44, 5/38**	*P = 0.003 (trend), **P = 0.014 (life-table test)	
		Squamous cell papilloma or carcinoma (combined) 2/45*, 2/42, 3/44, 5/38**	*P = 0.004 (trend), **P = 0.014 (life-table test) One carcinoma in each benzene-treated group; one mouse in the high-dose group had papilloma and carcinoma	
		Liver Hepatocellular carcinoma 9/49, 8/48, 17/50*, 8/47	*P = 0.028 (life-table test)	
		Hepatocellular adenoma or carcinoma (combined) 15/49, 17/48, 22/50*, 11/47	*P = 0.029 (life-table test)	

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence and/or multiplicity of tumours	Significance	Comments
Full carcinogenicity Mouse, B6C3F$_1$ (F) 6.5–8.5 wk 103 wk NTP (1986)	Gavage Benzene, > 99.7% Corn oil 0, 25, 50, 100 mg/kg bw 5 d/wk, 103 wk 50, 50, 50, 50 30, 26, 24, 18	*Zymbal gland*: squamous cell carcinoma 0/43*, 0/32, 1/37, 3/31**	*P = 0.007 (trend), **P = 0.045 (life-table test)	Principal strengths: 2 yr bioassay; control and three dose groups; complete histopathology; well-conducted GLP study; studies in both male and female mice Principal limitations: see principal limitations for NTP (1986) male mice study The incidence of preneoplastic hyperplasia was increased in tumours of the Zymbal gland, ovary, Harderian gland, lung, haematopoietic system (bone marrow), forestomach, and adrenal gland Historical incidence of lymphoma at laboratory (mean ± SD): 22/99 (22.2%); historical incidence in NTP studies: 237/1187 (20.0 ± 8.7%). Historical incidence of ovarian tumours at laboratory: 0/100; historical incidence in NTP studies: no more than two ovarian tumours were present in any single control group. Historical incidence of forestomach squamous cell papilloma at laboratory (mean): 0/99; historical incidence in NTP studies: 7/1077 (0.6%)
		Haematopoietic and lymphoid tissues Lymphoma 15/49*, 24/45**, 24/50***, 20/49****	*P = 0.031 (trend), **P = 0.021, ***P = 0.025, ****P = 0.037 (life-table test)	
		Lymphoma or leukaemia (combined) 15/49*, 25/45**, 26/50***, 22/49****	*P = 0.014 (trend), **P = 0.014, ***P = 0.012, ****P = 0.017 (life-table test) Leukaemia in 1 low-dose, 2 intermediate-dose, and 2 high-dose mice	
		Lung Alveolar/bronchiolar adenoma 4/49*, 2/42, 5/50, 9/49**	*P = 0.003 (trend), **P = 0.011 (life-table test)	
		Alveolar/bronchiolar carcinoma 0/49*, 3/42, 6/50**, 6/49***	*P = 0.002 (trend), **P = 0.010, ***P = 0.004 (life-table test)	
		Alveolar/bronchiolar adenoma or carcinoma (combined) 4/49*, 5/42, 10/50**, 13/49***	*P < 0.001 (trend), **P = 0.039, ***P < 0.001 (life-table test)	
		Ovary Tubular adenoma 0/47*, 0/44, 3/49, 3/48**	*P = 0.008 (trend), **P = 0.047 (life-table test)	
		Granulosa cell tumour [benign] 1/47*, 1/44, 6/49**, 7/48***	*P < 0.001 (trend), **P = 0.040, ***P = 0.008 (life-table test)	

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence and/or multiplicity of tumours	Significance	Comments
Full carcinogenicity Mouse, B6C3F$_1$ (F) 6.5–8.5 wk 103 wk NTP (1986) (cont.)		Granulosa cell tumour or carcinoma (combined) 1/47*, 1/44, 6/49**, 8/48*** Mixed tumour, benign 0/47*, 1/44, 12/49**, 7/48*** *Mammary gland* Carcinoma 0/49*, 2/45, 5/50**, 10/49*** Carcinosarcoma 0/49*, 0/45, 1/50, 4/49** *Harderian gland* Carcinoma 0/48*, 0/44, 0/50, 4/47** Adenoma or carcinoma (combined) 5/48*, 6/44, 10/50, 10/47** *Forestomach*: squamous cell papilloma 1/42*, 3/40, 6/45**, 5/42*** *Liver* Hepatocellular adenoma 1/49, 8/44*, 5/50, 4/49 Hepatocellular adenoma or carcinoma (combined) 4/49, 12/44*, 13/50**, 7/49	*P < 0.001 (trend), **P = 0.040, ***P = 0.004 (life-table test) One high-dose group mouse had carcinoma *P < 0.001 (trend), **P < 0.001, ***P = 0.001 (life-table test) *P < 0.001 (trend), **P = 0.026, ***P < 0.001 (life-table test) *P < 0.001 (trend), **P = 0.017 (life-table test) *P < 0.001 (trend), **P = 0.020 (life-table test) *P = 0.009 (trend), **P = 0.017 (life-table test) *P = 0.022 (trend), **P = 0.038, ***P = 0.040 (life-table test) *P = 0.008 (life-table test) *P = 0.014, **P = 0.008 (life-table test)	

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence and/or multiplicity of tumours	Significance	Comments
Full carcinogenicity Mouse, Swiss (M) 7 wk Lifetime Maltoni et al. (1988)	Gavage Benzene, 99.93% Olive oil 0, 500 mg/kg bw 4–5 d/wk, 78 wk 40, 40 NR	*Lung*: all tumours [mainly adenomas] 3/40, 17/40* *All sites* "Benign and malignant tumours" 15/40, 24/40* "Malignant tumours" 9/40, 14/40	*[$P < 0.001$][a] *[$P < 0.04$][b] [NS]	Principal strengths: lifetime study; studies in male and female mice Experiment BT 908; also reported in Maltoni et al. (1989)
Full carcinogenicity Mouse, Swiss (F) 7 wk Lifetime Maltoni et al. (1988)	Gavage Benzene, 99.93% Olive oil 0, 500 mg/kg bw 4–5 d/wk, 78 wk 40, 40 NR	*Mammary gland*: carcinoma 2/40, 19/40* *Lung*: all tumours [adenomas] 4/40, 15/40* *All sites* "Benign and malignant tumours" 16/40, 32/40* "Malignant tumours" 11/40, 28/40*	*[$P < 0.0001$][a] *[$P < 0.01$][a] *[$P \leq 0.0005$][a] *[$P \leq 0.0003$][a]	Principal strengths: lifetime study; studies in male and female mice Experiment BT 908; also reported in Maltoni et al. (1989)
Full carcinogenicity Mouse, RF/J (M) 6 wk Lifetime Maltoni et al. (1989)	Gavage Benzene, 99.93% Olive oil 0, 500 mg/kg bw 4–5 d/wk, 52 wk 45, 45 NR	*Lung*: all tumours [adenomas] 5/45, 23/45* *Haematopoietic and lymphoid tissues*: leukaemia 17/45, 26/45* *All sites* "Benign and malignant tumours" 18/45, 33/45* "Malignant tumours" 19/45, 26/45	*[$P < 0.0001$][a] *[$P < 0.05$][b] *[$P < 0.003$][a] [NS]	Principal strengths: lifetime study; studies in male and female mice Experiment BT 909

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence and/or multiplicity of tumours	Significance	Comments
Full carcinogenicity Mouse, RF/J (F) 6 wk Lifetime Maltoni et al. (1989)	Gavage Benzene, 99.93% Olive oil 0, 500 mg/kg bw 4–5 d/wk, 52 wk 40, 40 NR	*Mammary gland*: carcinoma 1/40, 9/40* *Haematopoietic and lymphoid tissues*: leukaemia 14/40, 24/40* *Lung*: all tumours [mainly adenomas] 3/40, 18/40* *All sites* "Benign and malignant tumours" 20/40, 34/40* "Malignant tumours" 3/40, 18/40*	*[$P < 0.02$][a] *[$P < 0.05$][a] *[$P < 0.0002$][a] *[$P < 0.002$][a] *[$P \leq 0.0002$][a]	Principal strengths: lifetime study; studies in male and female mice Experiment BT 909
Full carcinogenicity Mouse, A/J (M) 6–8 wk 24 wk Stoner et al. (1986)	Gavage Benzene, purity NR (reagent grade) Tricaprylin 0, 100 mg/kg bw 3×/wk for 8 wk 16, 16 15, 16	*Lung*: adenoma 3/15, 8/16 Tumour multiplicity: 0.27 ± 0.59, 0.63 ± 0.72*	 NR [NS] *$P < 0.05$	Principal limitations: incomplete histopathology reporting; only one dose group Cumulative dose was 2400 mg/kg bw, or 100 mg/kg bw/dose, as animals were gavaged 3×/wk for 8 wk
Full carcinogenicity Mouse, A/J (F) 6–8 wk 24 wk Stoner et al. (1986)	Gavage Benzene, purity NR (reagent grade) Tricaprylin 0, 100 mg/kg bw 3×/wk for 8 wk 16, 16 14, 15	*Lung*: adenoma 2/14, 5/15 Tumour multiplicity: 0.14 ± 0.36, 0.53 ± 0.92	 NR [NS] NS	Principal limitations: incomplete histopathology reporting; only one dose group Cumulative dose was 2400 mg/kg bw or 100 mg/kg bw/dose, as animals were gavaged 3×/wk for 8 wk

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence and/or multiplicity of tumours	Significance	Comments
Full carcinogenicity Mouse, A/J (M) 6–8 wk 24 wk Stoner et al. (1986)	i.p. Benzene, purity NR (reagent grade) Tricaprylin 0, 20, 50, 100 mg/kg bw 3×/wk for 8 wk 16, 16, 16, 16 16, 15, 16, 16	*Lung*: adenoma 3/16, 5/15, 8/16, 10/16* Tumour multiplicity: 0.25 ± 0.58, 0.53 ± 0.92, 0.63 ± 0.72*, 0.69 ± 0.60*	*[$P < 0.03$] *$P < 0.05$	Principal limitations: incomplete histopathology reporting Cumulative doses were 480, 1200, and 2400 mg/kg bw, or 20, 50, and 100 mg/kg bw, as animals were i.p. injected 3×/wk for 8 wk
Full carcinogenicity Mouse, A/J (F) 6–8 wk 24 wk Stoner et al. (1986)	i.p. Benzene, purity NR (reagent grade) Tricaprylin 0, 20, 50, 100 mg/kg bw 3×/wk for 8 wk 16, 16, 16, 16 16, 16, 16, 15	*Lung*: adenoma 4/16, 4/16, 4/16, 6/15 Tumour multiplicity: 0.31 ± 0.60, 0.44 ± 0.89, 0.25 ± 0.45, 0.47 ± 0.64	NR [NS] NS	Principal limitations: incomplete histopathology reporting Cumulative doses were 480, 1200, and 2400 mg/kg bw, or 20, 50, and 100 mg/kg bw, as animals were i.p. injected 3×/wk for 8 wk
Full carcinogenicity Mouse, CD-1 (M) In utero 12 mo Badham et al. (2010)	i.p. Benzene, purity NR Corn oil 0, 200, 400 mg/kg bw to pregnant dams on gestation days 8, 10, 12, and 14 25, 25, 25 NR	*All sites*: total tumours 6/22, 14/22*, 8/23 *Liver tumours* [primarily adenomas] 3/22, 10/22*, 4/23	*$P = 0.0329$[a] *$P = 0.0452$[a]	Transplacental carcinogenesis The authors also reported an experiment in male C57BP/6N mice with a similar study design, which gave negative results

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence and/or multiplicity of tumours	Significance	Comments
Full carcinogenicity Mouse, CD-1 (F) In utero 12 mo Badham et al. (2010)	i.p. Benzene, purity NR Corn oil 0, 200, 400 mg/kg bw to pregnant dams on gestation days 8, 10, 12, and 14 25, 25, 25 NR	*All sites*: total tumours 1/25, 10/24*, 5/22 Lesions of the haematopoietic and lymphoid tissues: hyperplasias, myeloproliferative disorders, and myeloid/lymphoid neoplasias 1/25, 9/24*, 5/22	*P = 0.0019[a] *P = 0.0232[a]	Transplacental carcinogenesis The authors also reported an experiment in female C57BP/6N mice with a similar study design, which gave negative results

bw, body weight; d, day(s); F, female; GLP, good laboratory practice; i.p., intraperitoneal; M, male; mo, month(s); NOS, not otherwise specified; NR, not reported; NS, not significant; NTP, National Toxicology Program; SD, standard deviation; wk, week(s); yr, year(s)

[a] Fisher exact test

[b] One-tail Fisher exact test

in males and/or females, and of carcinoma of the mammary gland in females (2/40 vs 19/40). Carcinoma of the Zymbal gland occurred in four males and one female and dysplasia of the Zymbal gland in three males and four females, compared with none in controls (Maltoni et al., 1988, 1989). [The Working Group noted that this lifetime study did not report on numerical body weight data, statistical analyses, or incidence of benign tumours of the mammary gland.]

A group of 45 male and 40 female RF/J mice (age, 6 weeks) was given benzene (purity, > 99.93%) at a dose of 0 (control) or 500 mg/kg bw in olive oil by stomach tube once per day, 4–5 days per week, for 52 weeks. Mice were observed for life. Necropsies were performed on all animals, with histopathological examinations on 29 tissues and organs (several with multiple sections, e.g. gastrointestinal tract), and all lesions. There were significant increases in the incidence of primary tumours (benign and malignant) (male, 18/45 vs 33/45; female, 20/40 vs 34/40), malignant tumours (female, 3/40 vs 18/40), total tumours [mainly adenomas] of the lung (male, 5/45 vs 23/45; female, 3/40 vs 18/40), and leukaemia (male, 17/45 vs 26/45; female, 14/40 vs 24/40) in males and/or females, and of carcinoma of the mammary gland in females (1/40 vs 9/40) (Maltoni et al., 1989). [The Working Group noted that this lifetime study did not report on body weights, statistical analyses, or incidence of benign tumours of the mammary gland.]

Groups of 16 male and 16 female A/J mice (age, 6–8 weeks) were given benzene (reagent grade) [purity not reported] at a dose of 0 (control) or 100 mg/kg bw [reported as cumulative dose of 2400 mg/kg bw] in 0.1 mL of tricaprylin per mouse, by gavage 3 days per week, for 24 weeks. Of the male mice, 15/16 (control) versus 16/16 (exposed to benzene) survived; for female mice, 14/16 (control) versus 15/16 (exposed to benzene) survived. At necropsy, tumours of the lung (pearly white nodules on the surface of the lungs) were counted under a dissecting microscope. Random samples of lung nodules were taken from control groups and groups treated with benzene for histopathological evaluation and confirmation of adenoma of the lung. The liver, kidneys, spleen, intestines, stomach, thymus, and salivary and endocrine glands were also examined grossly. The incidence of adenoma of the lung was numerically increased, but not significantly (males, 3/15 vs 8/16; females, 2/14 vs 5/15). Lung adenoma multiplicity was increased in exposed males (0.27 ± 0.59 vs 0.63 ± 0.72, $P < 0.05$) but not in exposed females (0.14 ± 0.36 vs 0.53 ± 0.92) (Stoner et al., 1986). [The Working Group noted that benzene purity was not reported, the histopathological report was incomplete, and only one dose was tested.]

3.1.3 *Intraperitoneal injection*

See Table 3.2

Groups of 16 male and 16 female A/J mice (age, 6–8 weeks) were given benzene (reagent grade) [purity not reported] at a dose of 0 (control), 20, 50, and 100 mg/kg bw [reported as cumulative doses of 480, 1200, and 2400 mg/kg bw] in 0.1 mL tricaprylin per mouse, by intraperitoneal injection, 3 days per week, for 24 weeks. One male from the group given the lowest dose and one female from the group given the highest dose died early. At necropsy, tumours of the lung (pearly white nodules on the surface of the lungs) were counted under a dissecting microscope. Random samples of lung nodules were taken from control groups and groups treated for benzene for histopathological evaluation and confirmation of adenoma of the lung. Liver, kidneys, spleen, intestines, stomach, thymus, and salivary and endocrine glands were also examined grossly. The incidence of adenoma of the lung was significantly increased in males given the highest dose (3/16 vs 5/15, 8/16, and 10/16 [$P < 0.03$]), as was multiplicity of adenoma of the lung in males given medium and high doses (0.25 ± 0.58 vs 0.53 ± 0.92, 0.63 ± 0.72 ($P < 0.05$), and 0.69 ± 0.60 ($P < 0.05$)). Neither

lung tumour incidence (4/16 vs 4/16, 4/16, and 6/15) nor multiplicity (0.31 ± 0.60 vs 0.44 ± 0.89, 0.25 ± 0.45, and 0.47 ± 0.64) were significantly increased in treated females (Stoner et al., 1986). [The Working Group noted the incomplete histopathological report.]

CD-1 and C57Bl/6N male and female mice (age, 7–9 weeks) were acclimated for 1 week before use, and given access to rodent chow and tap water. A maximum of 3 females were housed with 1 male overnight, and vaginal plugs the next morning designated day 1 of gestation. Pregnant mice were given corn oil (vehicle) or benzene at a dose of 200 or 400 mg/kg bw [purity not reported] by intraperitoneal injection on days 8, 10, 12, and 14 of gestation (Badham et al., 2010). At an age of 1 year, offspring mice were killed, necropsied, and tissues or organs collected (i.e. heart, intestines, kidneys, liver, lung, spine, spleen, stomach, thymus, and any abnormal tissues). Blinded histopathology was performed under light microscopy.

All tumours observed originated from the lung, liver, or haematopoietic and lymphoid tissues. CD-1 mice exposed in utero to benzene at 200 mg/kg bw had significant increases in the incidence of total tumours (combined), while the group exposed to benzene at 400 mg/kg bw had non-significant numerical increases only: males, 6/22 (27.3%) vs 14/22 (63.6%; $P = 0.0329$, Fisher exact test) and 8/23 (34.8%); females, 1/25 (4.0%) vs 10/24 (41.7%; $P = 0.0019$, Fisher exact test) and 5/22 (22.7%). In C57Bl/6N mice, there was a low incidence of total tumours (combined) only in groups treated with benzene: males, 0/21 vs 1/22 (4.5%) and 1/25 (4.0%); females, 0/19 vs 3/20 (15.0%) and 2/22 (9.1%). CD-1 male mice given the low dose had significant increases in the incidence of tumours of the liver (primarily adenomas, with some focal nodular hyperplasias and carcinomas): 3/22 (13.6%) vs 10/22 (45.5%, $P = 0.0452$) and 4/23 (17.4%). CD-1 female mice given the low dose had significant increases in the incidence of tumours [lesions] of the haematopoietic and lymphoid tissues (hyperplasias, myeloproliferative disorders, or myeloid/lymphoid neoplasias, combined): 1/25 (4.0%) vs 9/24 (37.5%, $P = 0.0232$) and 5/22 (22.7%). In C57Bl/6N mice, there was a low incidence of tumours [lesions] of the haematopoietic and lymphoid tissues only in the groups treated with benzene: males, 0/21 vs 0/22 and 1/25 (4.0%); females, 0/19 vs 3/20 (15.0%) and 2/22 (9.1%) (Badham et al., 2010). [The Working Group noted that dams were not carried to the end of the study for possible tumour occurrence, and that the study did not report benzene purity, injection volumes, beginning and ending body weights, and numbers of surviving mice. The Working Group also noted the short study duration of 12 months, that not all tissues or organs were taken or examined microscopically, and that tumours of the haematopoietic and lymphoid tissues were lesions grouped as hyperplasias, myeloproliferative disorders, and myeloid/lymphoid neoplasias.]

3.2 Rat

See Table 3.3

3.2.1 Oral administration

Groups of 30–35 male and 30–35 female Sprague-Dawley rats (age, 13 weeks) were given benzene (purity, > 99.9%) at a dose of 0 (control), 50, or 250 mg/kg bw in olive oil by gavage once per day, 4 or 5 days per week, for 52 weeks (Maltoni & Scarnato, 1979; Maltoni et al., 1983; see also Maltoni & Scarnato, 1977; Maltoni et al., 1985, 1989; Mehlman, 2002) [experiment BT901]. The rats were kept under observation for their lifespan. Mortality, correlated with the direct toxic effects and the higher incidence of malignant tumours, was higher in male and female rats treated with benzene. In treated males, a significant increase in the incidence of tumours of the haematopoietic and lymphoid

Table 3.3 Studies of carcinogenicity in rats exposed to benzene

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Rat, Sprague-Dawley (M) 13 wk Lifetime Maltoni & Scarnato (1979), Maltoni et al. (1983)	Gavage Benzene, > 99.9% Olive oil 0, 50, 250 mg/kg bw 1×/d, 4–5 d/wk, 52 wk 30, 30, 35 NR	*Zymbal gland*: carcinoma 0/30, 0/30, 0/35 *Oral cavity*: carcinoma 0/30, 0/30, 0/35 *Haematopoietic and lymphoid tissues*: haemolymphoreticular neoplasia (lymphoma, leukaemia, or histiocytic sarcoma, combined) 0/30*, 0/30, 4/35 (11.4%) *Mammary gland*: carcinoma 0/30, 0/30, 0/35 *All sites*: malignant tumours 2/30 (6.7%)*, 1/30 (3.3%), 8/35 (22.9%)	[NS] [NS] *[$P = 0.033$] (Cochran–Armitage trend test) [NS] *[$P = 0.025$] (Cochran–Armitage trend test)	Principal strengths: lifetime study; studies in male and female mice; multiple-dose study; complete histopathology Principal limitations: mortality was higher in benzene-treated rats Experiment BT901
Full carcinogenicity Rat, Sprague-Dawley (F) 13 wk Lifetime Maltoni & Scarnato (1979), Maltoni et al. (1983)	Gavage Benzene, > 99.9% Olive oil 0, 50, 250 mg/kg bw 1×/d, 4–5 d/wk, 52 wk 30, 30, 35 NR	*Zymbal gland*: carcinoma 0/30*, 2/30 (6.7%), 8/35 (22.9%)** *Oral cavity*: carcinoma 0/30, 0/30, 2/35 (5.7%) *Haematopoietic and lymphoid tissues*: haemolymphoreticular neoplasia (lymphoma, leukaemia, or histiocytic sarcoma, combined) 1/30 (3.3%), 2/30 (6.7%), 1/35 (3%) *Mammary gland*: carcinoma 3/30 (10%), 4/30 (13%), 7/35 (20.0%) *All sites*: malignant tumours 6/30* (20.0%), 10/30 (33.3%), 21/35 (60.0%)	*[$P = 0.005$] (Cochran–Armitage trend test), **[$P = 0.006$] (Fisher exact test) [NS] [NS] [NS]	Principal strengths: lifetime study; studies in male and female mice; multiple-dose study; complete histopathology Principal limitations: mortality was higher in benzene-treated rats Experiment BT901

Table 3.3 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Rat, Sprague-Dawley (M) 7 wk Lifetime Maltoni et al. (1982a, 1983, 1985)	Gavage Benzene, > 99.9% Olive oil 0, 500 mg/kg bw 1×/d, 4–5 d/wk, 104 wk 50, 40 NR	*Zymbal gland*: carcinoma 1/50 (2.0%), 18/40 (45.0%)*	*[$P < 0.001$] (Fisher exact test)	Principal strengths: lifetime study; studies in male and female mice; complete histopathology Principal limitations: benzene-treated rats had lower body weights Experiment BT902
		Oral cavity: squamous cell carcinoma 0/50, 21/40 (52.5%)*	*[$P < 0.001$] (Fisher exact test)	
		Nasal cavity: carcinoma 0/50, 3/40 (7.5%)	[NS]	
		Forestomach Acanthomas and dysplasias 0/50, 10/40 (25.0%)*	*[$P < 0.001$] (Fisher exact test)	
		Carcinoma in situ 0/50, 0/40	[NS]	
		Liver Hepatoma [hepatocellular carcinoma] 3/50 (6.0%), 3/40 (7.5%)	[NS]	
		Angiosarcoma 0/50, 2/40 (5.0%)	[NS]	
		Haematopoietic and lymphoid tissues: haemolymphoreticular neoplasia 3/50 (6.0%), 1/40 (2.5%)	[NS]	
		Skin: carcinoma 0/50, 9/40 (22.5%)*	*[$P < 0.001$] (Fisher exact test)	
		All sites: malignant tumours 11/50 (22.0%), 36/40 (90.0%)*	*[$P < 0.001$] (Fisher exact test)	

Table 3.3 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Rat, Sprague-Dawley (F) 7 wk Lifetime Maltoni et al. (1982a, 1983, 1985)	Gavage Benzene, > 99.9% Olive oil 0, 500 mg/kg bw 1×/d, 4–5 d/wk, 104 wk 50, 40 NR	*Zymbal gland*: carcinoma 0/50, 16/40 (40.0%)* *Oral cavity*: squamous carcinoma 0/50, 20/40 (50.0%)* *Nasal cavity*: carcinoma 0/50, 1/40 (2.5%) *Forestomach* Acanthomas and dysplasias 0/50, 7/40 (17.5%)* Carcinoma in situ 0/50, 6/40 (15.0%)* *Liver* Hepatoma [hepatocellular carcinoma] 0/50, 1/40 (2.5%) Angiosarcoma 0/50, 3/40 (7.5%) *Haematopoietic and lymphoid tissues*: haemolymphoreticular neoplasia 1/50 (2.0%), 3/40 (7.5%) *Skin*: carcinoma 1/50 (2.0%), 0/40 *All sites*: malignant tumours 10/50 (20.0%), 35/40 (87.5%)*	*[$P < 0.001$] (Fisher exact test) *[$P < 0.001$] (Fisher exact test) [NS] *[$P = 0.002$] (Fisher exact test) *[$P = 0.006$] (Fisher exact test) [NS] [NS] [NS] [NS] *[$P < 0.001$] (Fisher exact test)	Principal strengths: lifetime study; studies in both male and female mice; complete histopathology Principal limitations: benzene-treated rats had lower body weights Experiment BT902

Table 3.3 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Rat, Wistar (M) 7 wk Lifetime Maltoni et al. (1988, 1989)	Gavage Benzene, > 99.9% Olive oil 0, 500 mg/kg bw 1×/d, 4–5 d/wk, 104 wk 40, 40 NR	*Zymbal gland*: carcinoma 0/40, 7/40 (17.5%)* *Oral cavity*: carcinoma 1/40 (2.5%), 2/40 (5.0%) *Nasal cavity*: carcinoma 0/40, 2/40 (5.0%) *Haematopoietic and lymphoid tissues*: haemolymphoreticular neoplasia 1/40 (2.5%), 2/40 (5.0%) *All sites*: malignant tumours 8/40 (20.0%), 19/40 (47.5%)*	*[$P = 0.012$] (Fisher exact test) [NS] [NS] [NS] *[$P = 0.017$] (Fisher exact test)	Principal strengths: lifetime study; studies in male and female mice; complete histopathology Principal limitations: benzene-treated rats had lower body weights; mortality was higher in benzene-treated rats Experiment BT907
Full carcinogenicity Rat, Wistar (F) 7 wk Lifetime Maltoni et al. (1988, 1989)	Gavage Benzene, > 99.9% Olive oil 0, 500 mg/kg bw 1×/d, 4–5 d/wk, 104 wk 40, 40 NR	*Zymbal gland*: carcinoma 0/40, 6/40 (15.0%)* *Oral cavity*: carcinoma 0/40, 4/40 (10.0%) *Nasal cavity*: carcinoma 0/40, 1/40 (2.5%) *Haematopoietic and lymphoid tissues*: haemolymphoreticular neoplasia 3/40 (7.5%), 4/40 (10.0%) *All sites*: malignant tumours 10/40 (25.0%), 21/40 (52.5%)*	*[$P = 0.026$] (Fisher exact test) [NS] [NS] [NS] *[$P = 0.021$] (Fisher exact test)	Principal strengths: lifetime study; studies in both male and female mice; complete histopathology Principal limitations: benzene-treated rats had lower body weights; mortality was higher in benzene-treated rats Experiment BT907

Table 3.3 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Rat, F344/N (M) 7–8 wk 104 wk NTP (1986)	Gavage Benzene, > 99.7% Corn oil 0, 50, 100, 200 mg/kg bw 1×/d, 5 d/wk, 103 wk 50, 50, 50, 50 32, 29, 24, 16	*Zymbal gland*: carcinoma 2/32 (6.3%)*, 6/46 (13.0%), 10/42 (23.8%)**, 17/42 (40.5%)***	*$P < 0.001$[a], $P < 0.001$[c], $P = 0.003$[d] **$P = 0.040$[b], $P = 0.017$[c] ***$P < 0.001$[b], $P < 0.001$[c], $P = 0.024$[d]	Principal strengths: studies in male and female mice; multiple-dose study; covered most of the lifespan; complete histopathology; well-conducted GLP study Principal limitations: benzene-treated rats had lower body weights; mortality was higher in benzene-treated rats Dose-related lymphocytopenia was observed in benzene-treated rats Statistical tests used: [a] Cochran–Armitage trend test; [b] Fisher exact test; [c] Life-table test; [d] Incidental tumour test
		Oral cavity Squamous cell carcinoma 0/50*, 3/50, 5/50**, 7/50***	*$P = 0.006$[a], $P = 0.001$[c], $P = 0.002$[d] **$P = 0.028$[b], $P = 0.030$[c] ***$P = 0.006$[b], $P = 0.001$[c], $P = 0.001$[d]	
		Squamous cell papilloma 1/50*, 6/50, 11/50**, 13/50***	*$P < 0.001$[a], $P < 0.001$[b], $P < 0.001$[d] **$P = 0.002$[b], $P = 0.001$[c], $P = 0.004$[d] ***$P < 0.001$[b], $P < 0.001$[c], $P = 0.002$[d]	
		Tongue: squamous cell carcinoma 0/50*, 3/50, 4/50, 4/50**	*NS[a], $P = 0.039$[c], $P = 0.049$[d] **$P = 0.028$[c], $P = 0.012$[d]	
		Lip: squamous cell carcinoma 0/50*, 0/50, 0/50, 3/50**	*$P = 0.012$[a], $P = 0.002$[c], $P = 0.008$[d] **$P = 0.035$[c]	
		Skin Squamous cell carcinoma 0/50*, 5/50**, 3/50, 8/50***	*$P = 0.007$[a], $P < 0.001$[c] **$P = 0.028$[b], $P = 0.032$[c] ***$P = 0.003$[b], $P = 0.001$[c], $P = 0.039$[d]	

Table 3.3 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Rat, F344/N (M) 7–8 wk 104 wk NTP (1986) (cont.)		Squamous cell papilloma 0/50*, 2/50, 1/50, 5/50**	*$P = 0.014$[a], $P = 0.001$[c], $P = 0.002$[d] **$P = 0.028$[b], $P = 0.005$[c], $P = 0.009$[d]	
		Palate Squamous cell papilloma 0/50*, 4/50, 4/50, 9/50**	*$P = 0.002$[a], $P < 0.001$[c], $P = 0.005$[d] **$P = 0.001$[b], $P < 0.001$[c], $P = 0.006$[d]	
		Squamous cell carcinoma 0/50, 0/50, 1/50, 0/50	NS	
Full carcinogenicity Rat, F344/N (F) 7–8 wk 104 wk NTP (1986)	Gavage Benzene, > 99.7% Corn oil 0, 25, 50, 100 mg/kg bw 1×/d, 5 d/wk, 103 wk 50, 50, 50, 50 46, 38, 33, 25	*Zymbal gland*: carcinoma 0/45*, 5/40**, 5/44***, 14/46 (30.4%)****	*$P < 0.001$[a], $P < 0.001$[c], $P < 0.001$[d] **$P = 0.020$[b], $P = 0.022$[c], $P = 0.036$[d] ***$P = 0.026$[b], $P = 0.018$[c] ****$P < 0.001$[b], $P < 0.001$[c], $P < 0.001$[d]	Principal strengths: studies in male and female mice; multiple-dose study; covered most of the lifespan; complete histopathology; well-conducted GLP study Principal limitations: benzene-treated rats had lower body weights; mortality was higher in benzene-treated rats Dose-related lymphocytopenia was observed in benzene-treated rats; historical incidence of endometrial stromal polyps: 22/98 (22.4%) at laboratory; 248/1125 (22.0 ± 7%) (4/49–17/50) overall Statistical tests used: [a] Cochran–Armitage trend test; [b] Fisher exact test; [c] Life-table test; [d] Incidental tumour test
		Oral cavity Squamous cell carcinoma 0/50*, 1/50, 4/50, 5/50**	*$P = 0.011$[a], $P = 0.003$[c] **$P = 0.028$[b], $P = 0.010$[c]	
		Squamous cell papilloma 1/50*, 4/50, 8/50**, 5/50***	*NS[a], $P = 0.017$[c], $P = 0.047$[d] **$P = 0.015$[b], $P = 0.006$[c], $P = 0.022$[d] ***$P = 0.032$[c]	

Table 3.3 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Rat, F344/N (F) 7–8 wk 104 wk NTP (1986) (cont.)		*Tongue*: squamous cell carcinoma 0/50*, 0/50, 4/50**, 4/50***	*P = 0.014[a], P = 0.004[c] **P = 0.047[c] ***P = 0.024[c]	
		Uterus: endometrial stromal polyp 7/50* (14.0%), 7/50 (14.0%), 7/49 (14.3%), 14/50 (28.0%)**	*P = 0.032[a], P = 0.001[c], P = 0.049[d] **P = 0.003[c], P = 0.049[d]	
Full carcinogenicity Rat, Sprague-Dawley (F) 13 wk (breeders) Lifetime Maltoni et al. (1983, 1985, 1989)	Inhalation Benzene, > 99.9% Air 0, 200–300 ppm 4–7 h/d, 5 d/wk, 104 wk 60, 54 NR	*Zymbal gland*: carcinoma 1/60 (1.7%), 3/54 (5.6%)	[NS]	Principal strengths: lifetime study; complete histopathology Principal limitations: breeders were aged 13 wk at the start of exposure Experiment BT4004, BT4006; benzene-treated rats presented lymphocytopenia
		Oral cavity: carcinoma 0/60, 2/54 (3.7%)	[NS]	
		Forestomach: carcinoma in situ 0/60, 0/54	[NS]	
		Liver: hepatoma [hepatocellular carcinoma] 0/60, 1/54 (1.9%)	[NS]	
		Mammary gland: malignant tumours 2/60 (3.3%), 6/54 (11.1%)	[NS]	
		Haematopoietic and lymphoid tissues: haemolymphoreticular neoplasia 2/60 (3.3%), 0/54	[NS]	
		All sites: malignant tumours 9/60 (15.0%), 14/54 (25.9%)	[NS]	

Table 3.3 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Rat, Sprague-Dawley (M) Embryo (gestation day 12) Lifetime Maltoni et al. (1983, 1985, 1989)	Inhalation Benzene, > 99.9% Air 0, 200, 200–300 ppm 4–7 h/d, 5 d/wk, 15 wk (200 ppm) or 104 wk (0 ppm or 200–300 ppm) 158, 70, 75 NR	*Zymbal gland*: carcinoma 2/158 (1.3%), 4/70 (5.7%), 6/75 (8.0%)*	*[$P = 0.015$] (Fisher exact test)	Principal strengths: lifetime study; multiple-dose study; studies in male and female mice; in utero exposure; complete histopathology Experiment BT4004, 4006; benzene-treated rats presented lymphocytopenia
		Oral cavity: carcinoma 0/158, 2/70 (2.9%), 1/75 (1.3%)	[NS]	
		Forestomach: carcinoma in situ 0/158, 0/70, 0/75	[NS]	
		Liver: hepatoma [hepatocellular carcinoma] 1/158 (0.6%), 2/70 (2.9%), 2/75 (2.7%)	[NS]	
		Mammary gland: malignant tumours 3/158 (1.9%), 0/70, 0/75	[NS]	
		Haematopoietic and lymphoid tissues: haemolymphoreticular neoplasia 12/158 (7.6%), 4/70 (5.7%), 6/75 (8.0%)	[NS]	
		All sites: malignant tumours 28/158 (17.7%), 20/70 (28.6%), 24/75 (32.0%)**	*[$P = 0.018$] (Fisher exact test)	

Table 3.3 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Rat, Sprague-Dawley (F) Embryo (gestation day 12) Lifetime Maltoni et al. (1983, 1985, 1989)	Inhalation Benzene, > 99.9% Air 0, 200, 200–300 ppm 4–7 h/d, 5 d/wk, 15 wk (200 ppm) or 104 wk (0 ppm or 200–300 ppm) 149, 59, 65 NR	*Zymbal gland*: carcinoma 0/149, 1/59 (1.7%), 8/65 (12.3%)*	*[$P < 0.001$] (Fisher exact test)	Principal strengths: lifetime study; multiple-dose study; studies in male and female mice; in utero exposure; complete histopathology Experiment BT4004, 4006; benzene-treated rats presented lymphocytopenia
		Oral cavity: carcinoma 0/149, 6/59 (10.2%)*, 10/65 (15.4%)**	*[$P < 0.001$] **[$P < 0.001$] (Fisher exact test)	
		Forestomach: carcinoma in situ 0/149, 0/59, 3/65 (4.6%)*	*[$P = 0.027$] (Fisher exact test)	
		Liver: hepatoma [hepatocellular carcinoma] 0/149, 5/59 (8.5%)*, 7/65 (10.8%)**	*[$P = 0.002$] **[$P < 0.001$] (Fisher exact test)	
		Mammary gland: malignant tumours 8/149 (5.4%), 8/59 (13.6%), 9/65 (13.8%)	[NS]	
		Haematopoietic and lymphoid tissues: haemolymphoreticular neoplasia 1/149 (0.7%), 4/59 (6.8%)*, 0/65	*[$P = 0.024$] (Fisher exact test)	
		All sites: malignant tumours 26/149 (17.4%), 26/59 (44.1%)*, 38/65 (58.5%)**	*[$P < 0.001$] **[$P < 0.001$] (Fisher exact test)	

bw, body weight; d, day(s); F, female; GLP, good laboratory practice; h, hour(s); M, male; NR, not reported; NS, not significant; ppm, parts per million; wk, week

tissues (lymphoma, leukaemia, or histiocytic sarcoma, combined) was observed: 0/30, 0/30, and 4/35 (11%) [P = 0.033, Cochran–Armitage trend test]. A significant dose-related increase in the incidence of carcinoma of the Zymbal gland was observed in treated female rats: 0/30, 2/30 (7%), and 8/35 (23%) [P = 0.006, Fisher exact test; P = 0.005, Cochran–Armitage trend test]. The overall incidence of malignant tumours in male rats was 2/30 (7%), 1/30 (3%), and 8/35 (23%) [P = 0.025, Cochran–Armitage trend test]. The overall incidence of malignant tumours in female rats was 6/30 (20%), 10/30 (33%), and 21/35 (66%) [P = 0.003, Cochrane–Armitage trend test]. [The Working Group noted the small number of animals and the start of the treatment from age 13 weeks, slightly later than the usual 7–8 weeks. This might partly explain why certain malignant tumours increased in incidence, in particular carcinoma of the oral cavity and carcinoma of the mammary gland, but these increases were not statistically significant. The Working Group noted that the principal strengths of the study were: lifespan was covered; both male and female rats were studied; multiple doses tested; and complete histopathology. The principal limitation was that mortality was higher in rats treated with benzene.]

Groups of 40–50 male and 40–50 female Sprague-Dawley rats (age, 7 weeks) were given benzene (purity, > 99.9%) at a dose of 0 (control) or 500 mg/kg bw in olive oil by gavage once per day, 4 or 5 days per week, for 104 weeks (Maltoni et al., 1982a, 1983, 1985; see also Maltoni et al., 1989) [experiment BT902]. The rats were kept under observation for their lifespan. Male and female rats treated with benzene had lower body weights and showed lymphocytopenia. A significant increase in the incidence of carcinoma of the Zymbal gland was observed in treated male rats: 1/50 (2.0%) versus 18/40 (45.0%) [P < 0.001, Fisher exact test]. A significant increase in the incidence of carcinoma of the Zymbal gland was also observed in treated female rats: 0/50 versus 16/40 (40.0%) [P < 0.001, Fisher exact test]. A significantly increased incidence of squamous cell carcinoma of the oral cavity was observed in treated male rats: 0/50 versus 21/40 (53%) [P < 0.001, Fisher exact test]. The incidence of squamous cell carcinoma of the oral cavity was also significantly increased in treated female rats: 0/50 versus 20/40 (50.0%) [P < 0.001, Fisher exact test]. In treated female rats, a significant increase in the incidence of carcinoma in situ of the forestomach was reported: 0/50 versus 6/40 (15.0%) [P = 0.006, Fisher exact test]. The incidence of precancerous lesions of the forestomach (acanthomas and dysplasias) was significantly increased in treated male and female rats: 0/50 versus 10/40 (25.0%) [P < 0.001, Fisher exact test] and 0/50 versus 7/40 (17.5%) [P = 0.002, Fisher exact test]. In treated male rats, the incidence of carcinoma of the skin was significantly increased: 0/50 versus 9/40 (22.5%) [P < 0.001, Fisher exact test]. The incidence of a rare tumour (angiosarcoma of the liver) was non-significantly increased in both male and female rats: 0/50 versus 2/40 (5.0%) and 0/50 versus 3/40 (7.5%). The overall incidence of malignant tumours in male rats was 11/50 (22.0%) versus 36/40 (90.0%) [P < 0.001, Fisher exact test]. The overall incidence of malignant tumours in female rats was 10/50 (20.0%) versus 35/40 (87.5%) [P < 0.001, Fisher exact test]. [The Working Group noted that the principal strengths of the study included: lifespan covered; both male and female rats studied; and complete histopathology. The principal limitation was that rats treated with benzene had lower body weights.]

Groups of 40 male and 40 female Wistar rats (age, 7 weeks) were given benzene (purity, > 99.9%) at a dose of 0 (control) or 500 mg/kg bw in olive oil by gavage once per day, 4 or 5 days per week, for 104 weeks (Maltoni et al., 1988, 1989; see also Maltoni et al., 1985) [experiment BT907]. Rats were kept under observation for their lifespan. Mortality was higher and body weights lower in male and female rats treated with benzene.

A significantly increased incidence of carcinoma of the Zymbal gland was reported in treated male and female rats: 0/40 versus 7/40 (17.5%) [$P = 0.012$, Fisher exact test] and 0/40 versus 6/40 (15.0%) [$P = 0.026$, Fisher exact test]. The overall incidence of malignant tumours in male and female rats was 8/40 (20.0%) versus 19/40 (47.5%) [$P = 0.017$, Fisher exact test] and 10/40 (25.0%) versus 21/40 (52.5%) [$P = 0.021$, Fischer exact test]. [The Working Group noted the principal strengths: the study covered the lifespan; both male and female rats were studied; and complete histopathology was reported. The principal limitations were the lower body weights and higher mortality in rats treated with benzene.]

In a GLP study, groups of 50 male F344/N rats (age, 7–8 weeks) were given benzene (purity, > 99.7%) at a dose of 0 (control), 50, 100, or 200 mg/kg bw in corn oil by gavage once per day, 5 days per week, for 103 weeks. Groups of 50 female F344/N rats (age, 7 weeks) were given benzene (purity, > 99.7%) at a dose of 0 (control), 25, 50, or 100 mg/kg bw in corn oil by gavage once per day, 5 days per week, for 103 weeks (NTP, 1986; see also Maronpot, 1987; Huff et al., 1989). The rats were kept under observation for 104 weeks and then killed. Higher mortality, lower body weights, and dose-related lymphocytopenia were all observed in male and female rats treated with benzene. A significant increase in the incidence, and positive trend in the incidence, of carcinoma of the Zymbal gland was observed in treated male and female rats. A significant increase in the incidence, and positive trend in the incidence, of squamous cell carcinoma of the oral cavity was observed in treated male and female rats. A significant increase in the incidence, and positive trend in the incidence, of squamous cell papilloma of the oral cavity was observed in treated male rats. A significant increase in the incidence of squamous cell papilloma of the oral cavity was also reported in treated female rats. There was a significant increase in the incidence, and positive trend in the incidence, of squamous cell carcinoma of the lip in treated male rats. In male rats, a significant increase in the incidence of squamous cell carcinoma of the tongue was reported. In female rats, a significant positive trend in the incidence of squamous cell carcinoma of the tongue was reported. There was a significant increase in the incidence, and positive trend in the incidence, of squamous cell papilloma of the palate in treated male rats. In male rats, significant increases in the incidence, and positive trend in the incidence, of squamous cell carcinoma and of squamous cell papilloma of the skin were reported. In female rats, a significant positive trend in the incidence of stromal polyp of the endometrium was reported. [The Working Group noted that the principal strengths included: well-conducted GLP study; multiple-dose study; most of the lifespan covered; both male and female rats studied; and complete histopathology was reported. The principal limitations were the lower body weights and higher mortality in rats treated with benzene.]

3.2.2 Inhalation

One group of pregnant breeders (54 female Sprague-Dawley rats; age, 13 weeks) was exposed to benzene (purity, > 99.9%) at a concentration of 200 ppm by inhalation from day 12 of gestation for 4 hours per day, 5 days per week, for 7 weeks (Maltoni et al., 1983, 1985, 1989; see also Maltoni et al., 1982b, c) [experiments BT4004, BT4006]. The embryos were exposed transplacentally by inhalation during the prenatal period, and possibly by ingestion (via lactation) during weaning. After weaning, a first group of offspring (70 males and 59 females) was exposed to benzene at 200 ppm by inhalation for 7 hours per day, 5 days per week for 8 weeks (total duration of exposure to benzene, 15 weeks). A second group of offspring (75 males and 65 females) and the breeders were exposed to benzene at 200 ppm by inhalation for 7 hours per day, 5 days per week, for 12 weeks, then exposed to benzene at

300 ppm for 7 hours per day, 5 days per week, for 85 weeks (total duration of exposure to benzene, 104 weeks). All groups of animals were located in inhalation chambers. The breeders control group (60 females) and the offspring control group (158 males and 149 females) were not exposed to benzene (untreated controls exposed to filtered air). The rats were then kept under observation for their lifespan. Mortality was higher in groups of male and female offspring treated with benzene that had lymphocytopenia. No significant increase in tumour incidence was found in the breeders. The overall incidence of malignant tumours in breeders was 9/60 (15.0%, control) versus 14/54 (25.9%). [Breeders were treated starting from age 13 weeks, slightly later than the usual 7–8 weeks. This might partly explain why malignant tumours increased in incidence, particularly carcinoma of the Zymbal gland, carcinoma of the oral cavity, and malignant tumours of the mammary gland, but none of these increases were statistically significant.] In the offspring, a significant increase in the incidence of carcinoma of the Zymbal gland was observed in treated male and female rats: 2/158 (1.3%) versus 4/70 (5.7%) and 6/75 (8.0%) [P = 0.015, Fisher exact test], and 0/149 versus 1/59 (1.7%) and 8/65 (12.3%) [P < 0.001, Fisher exact test], respectively. In female offspring, a significant increase in the incidence of carcinoma of the oral cavity was observed: 0/149 versus 6/59 (10.2%) [P < 0.001, Fisher exact test] and 10/65 (15.4%) [P < 0.001, Fisher exact test]. A significant increase in the incidence of carcinoma in situ of the forestomach was observed in treated female offspring: 0/149 versus 0/59 and 3/65 (4.6%) [P = 0.027, Fisher exact test]. A significant increase in the incidence of liver hepatoma [hepatocellular carcinoma] was observed in treated female offspring: 0/149 versus 5/59 (8.5%) [P = 0.002, Fisher exact test] and 7/65 (10.8%) [P < 0.001, Fisher exact test]. A significant increase in the incidence of tumours of the haematopoietic and lymphoid tissues (haemolymphoreticular neoplasia) was observed in treated female offspring: 1/149 (0.7%) versus 4/59 (6.8%) [P = 0.024, Fisher exact test] and 0/65. The overall incidence of malignant tumours in male and female offspring was: 28/158 (17.7%) versus 20/70 (28.6%) and 24/75 (32.0%) [P = 0.018, Fisher exact test], and 26/149 (17.4%) versus 26/59 (44.1%) [P < 0.001, Fisher exact test] and 38/65 (58.5%) [P < 0.001, Fisher exact test]. [The Working Group noted the principal strengths of the study: multiple-dose study; lifespan was covered; both male and female rats studied; in utero exposure; and complete histopathology. The principal limitation was that breeders were aged 13 weeks at the start of exposure.]

3.3 Genetically modified animals

See Table 3.4

Genetically modified animals have been used for accelerated testing of chemicals for their carcinogenic potential under specific conditions of exposure (Tennant et al., 1995, 2001; French et al., 2001a). These models are focused primarily on heritable mutations in: (1) tumour-suppressor genes with loss of function (e.g. transformation protein 53 or *Trp53*, cyclin-dependent kinase 2a or *Cdnk2a* [splice variants produce either p16Ink4a or p19Arf proteins]) and/or (2) proto-oncogenes (e.g. *Hras* or *vHras*) with gain of function due to acquired mutations associated with cancer in humans and mouse models of human cancer. Susceptibility or predisposition to chemical carcinogenesis is based on acquired or heritable mutations in tumour-suppressor gene and/or proto-oncogene functional pathways, and other modifiers of cancer in genetically modified animal models that are consistent with known hallmarks of human cancer (Hanahan & Weinberg, 2000, 2011). Accelerated tests for carcinogenic potential have shown reliability and potential, but with limitations that must be carefully considered (Pritchard et al., 2003; Eastmond et al., 2013).

Table 3.4 Studies of carcinogenicity in genetically modified animals exposed to benzene

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence and/or multiplicity of tumours	Significance	Comments
Carcinogenicity with other modifying factor Mouse, C57BL/6 or h-Trx-Tg (NR) NR Lifetime Li et al. (2006)	Inhalation (whole-body exposure) Benzene, purity NR Air 0, 300, 0, 300 ppm 6 h/d, 5 d/wk, 26 wk 8, 10, 8, 13 NR	*Thymus*: lymphoma 0/8, 3/10, 0/8, 0/13	NS	Principal strengths: lifetime study Principal limitations: small numbers of animals used The h-Trx-Tg mouse overexpresses human thioredoxin; survival was higher in control mice than in benzene-treated mice
Carcinogenicity with other modifying factor Mouse, B6.CBA-*Trp53* ^{tm1Sia} (M) 8 wk Lifetime Kawasaki et al. (2009)	Inhalation (whole-body exposure) Benzene, purity NR Clean air 0, 33, 100, 300 ppm 6 h/d, 5 d/wk 24, 27, 25, 26 NR	*Haematopoietic and lymphoid tissues* Thymic lymphoma 0/24, 1/27, 4/25, 19/26* Non-thymic lymphoma 9/24, 10/27, 5/25, 2/26 Acute myelocytic leukaemia 0/24, 0/27, 0/25, 2/26	*$P < 0.05$ (Fisher exact test), [$P < 0.001$] (Cochran–Armitage trend test) NS (for an increase) NS	Principal strengths: lifetime study Genetically modified mouse model based on *Trp53* tumour-suppressor gene wildtype and null allele (*Trp53* haploinsufficiency) modifying factor; heterozygous wildtype and null allele (*Trp53* haploinsufficient) *Trp53* allelotype was used; moribund mice or mice presenting with masses or significant body weight loss were killed for gross and histopathological examination
Carcinogenicity with other modifying factor Mouse, C3.CBA-*Trp53*^{tm1Sia} (M) 8 wk Lifetime Kawasaki et al. (2009)	Inhalation (whole-body exposure) Benzene, purity NR Clean air 0, 100, 300 ppm 6 h/d, 5 d/wk 24, 24, 24 NR	*Haematopoietic and lymphoid tissues* Thymic lymphoma 1/24, 12/24*, 6/24* Non-thymic lymphoma 3/24, 6/24, 10/24* Acute myelocytic leukaemia 2/24, 2/24, 9/24*	*$P < 0.05$ (Fisher exact test), [$P = 0.001$] (Cochran–Armitage trend test) *$P < 0.05$ (Fisher exact test) *$P < 0.05$ (Fisher exact test), [$P = 0.016$] Cochran–Armitage trend test)	Principal strengths: lifetime study Genetically modified mouse model based on *Trp53* tumour-suppressor gene wildtype and null allele (*Trp53* haploinsufficiency) modifying factor; heterozygous wildtype and null allele (*Trp53* haploinsufficient) *Trp53* allelotype was used; moribund mice or mice presenting with masses or significant body weight loss were killed for gross and histopathological examination

Table 3.4 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence and/or multiplicity of tumours	Significance	Comments
Carcinogenicity with other modifying factor Mouse, B6.129-*Trp53*^{tm1Bra} N5 (M) 7–10 wk 26 wk French & Saulnier (2000), French et al. (2001b)	Gavage Benzene, purity NR Corn oil 0, 200 mg/kg bw, 5×/wk, 26 wk 20, 40 20, 39	*Subcutis*: sarcoma, NOS 0/20, 16/39* *Thymus*: lymphoma 0/20, 3/39 *Pancreas*: acinar cell carcinoma 0/20, 1/39 *Lung*: tumours 0/20, 0/22	*[$P \leq 0.004$] (Fisher exact test) LOH in 13/16 sarcomas tested NS LOH in 3/3 lymphomas tested NS NS	Genetically modified mouse model based on *Trp53* tumour-suppressor gene wildtype and null allele (*Trp53* haploinsufficiency) modifying factor (heterozygous); study terminated 1 d after exposure period
Carcinogenicity with other modifying factor Mouse, B6.129-*Trp53*^{tm1Bra} N5 (M) NR 26 wk Storer et al. (2001)	Gavage Benzene, purity NR Corn oil 0, 100 mg/kg bw 5–7×/wk 15 + 15, 15 + 15 NR	*Thymus* Lymphoma 0/30, 4/30 Atypical hyperplasia [preneoplastic lesion] 0/30, 7/30* *Subcutis*: sarcoma 1/30, 1/30 *Bone*: osteosarcoma 0/30, 1/30	[NS] *[$P \leq 0.01$] (Fisher exact test) NS NS	Genetically modified mouse model based on *Trp53* tumour-suppressor gene wildtype and null allele (*Trp53* haploinsufficiency) modifying factor (heterozygous); data (combined) extracted from use of benzene at a single dose as a positive control in two studies of another agent
Carcinogenicity with other modifying factor Mouse, B6.129-*Trp53*^{tm1Bra} N5 (F) NR 26 wk Storer et al. (2001)	Gavage Benzene, purity NR Corn oil 0, 100 mg/kg bw, 5–7×/wk 15 + 15, 15 + 15 NR	*Thymus* Lymphoma 1/30, 1/30 Atypical hyperplasia [preneoplastic lesion] 0/30, 2/30 *Subcutis*: sarcoma 1/30, 1/30 *Bone*: osteosarcoma 1/30, 0/30	NS NS NS NS	Genetically modified mouse model based on *Trp53* tumour-suppressor gene wildtype and null allele (*Trp53* haploinsufficiency) modifying factor (heterozygous); data (combined) extracted from use of benzene at a single dose as a positive control in two studies of another agent

Table 3.4 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence and/ or multiplicity of tumours	Significance	Comments
Carcinogenicity with other modifying factor Mouse, B6.129-*Cdkn2a*^{tm1Dep} (M) 7–8 wk 27 wk NTP (2007)	Gavage Benzene, > 99% Corn oil 0, 25, 50, 100, 200 mg/kg bw, 5×/wk 15, 15, 15, 15, 15 15, 15, 15, 15, 14	*Multiple organs*: malignant lymphoma 0/15, 0/15, 0/15, 0/15, 5/15*	*P = 0.021 (Fisher exact test), [P < 0.001] Cochran–Armitage trend test)	Genetically modified mouse model based on *Cdkn2a* tumour-suppressor gene wildtype and null allele (*Cdkn2a* haploinsufficiency) modifying factor (heterozygous); both *p16Ink4a* and *p19Arf* tumour-suppressor gene functions were haploinsufficient
		Bone marrow: atrophy 0/15, 0/15, 0/15, 10/15*, 12/15*	*P ≤ 0.01 (Fisher exact test), [P < 0.001] Cochran–Armitage trend test)	
		Mesentery, lymph node: atrophy 1/15, 2/15, 2/14, 13/15*, 13/15*	*P ≤ 0.01 (Fisher exact test), [P < 0.001] Cochran–Armitage trend test)	
Carcinogenicity with other modifying factor Mouse, B6.129-*Cdkn2a*^{tm1Dep} (F) 7–8 wk 27 wk NTP (2007)	Gavage Benzene, > 99% Corn oil 0, 25, 50, 100, 200 mg/kg bw, 5×/wk 15, 15, 15, 15, 15 15, 15, 15, 15, 15	*Multiple organs*: malignant lymphoma 0/15, 0/15, 0/15, 0/15, 0/15	NS	Genetically modified mouse model based on *Cdkn2a* umour-suppressor gene wildtype and null allele (*Cdkn2a* haploinsufficiency) modifying factor (heterozygous); both *p16Ink4a* and *p19Arf* tumour-suppressor gene functions were haploinsufficient
		Mesentery, lymph node: atrophy 0/15, 2/15, 3/15, 8/15*, 6/15*	*P ≤ 0.01 (Fisher exact test), [P = 0.005] Cochran–Armitage trend test)	
Carcinogenicity with other modifying factor Mouse, FVB/N-Tg.AC (v-Ha-*Ras*) (F) 7 wk 20 wk Spalding et al. (1999)	Skin application Benzene, purity NR Acetone, neat 0, 400, 800, 1600 µL/wk 5, 10, 10, 10 4, 8, 8, 8	*Skin*: squamous cell papilloma 3/5, 7/10, 8/10, 10/10*	*P < 0.05 (life-table test)	FVB/N-Tg.AC (v-Ha-*Ras*) transgene insert (two copies or homozygous state); to achieve the weekly dosage regimen, benzene was applied 2 d/wk; skin papilloma incidence in control mice due to fighting
		Tumour multiplicity: 1.4 ± 1.7, 7.0 ± 10.3, 10.6 ± 8.5*, 12.6 ± 10.3*	*P < 0.05 (Mann–Whitney U-test)	
Carcinogenicity with other modifying factor Mouse, FVB/N-Tg.AC (v-Ha-*Ras*) (M) 8 wk 26 wk Holden et al. (1998)	Skin application Benzene, purity NR Acetone, neat 0, 100, 150 µL, 3×/wk for 20 wk 10, 10, 10 9, 8, 10	*Skin*: squamous cell papilloma 0/10, 0/10, 3/10	NS	FVB/N mouse carrying a v-Ha-*Ras* transgene insert (single-copy or hemizygous state); acetone control group treated daily

Table 3.4 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence and/ or multiplicity of tumours	Significance	Comments
Carcinogenicity with other modifying factor Mouse, FVB/N-Tg.AC (v-Ha-*Ras*) (F) 8 wk 26 wk Holden et al. (1998)	Skin application Benzene, purity NR Acetone, neat 0, 100, 150 µL, 3×/wk, 20 wk 10, 10, 10 5, 9, 7	*Skin*: squamous cell papilloma 0/10, 1/10, 1/10	NS	FVB/N-Tg.AC (v-Ha-*Ras*) transgene insert (single-copy or hemizygous state); acetone control group treated daily
Carcinogenicity with other modifying factor Mouse, FVB/N-Tg.AC (v-Ha-*Ras*) (F) NR 32 wk French & Saulnier (2000)	Skin application Benzene, purity NR Acetone, neat 0, 450, 800 µL/wk, 20 wk NR	*Bone marrow*: leukaemia, granulocytic 0/19, 4/14*, 11/15*	*$P < 0.05$ (Fisher exact test)	Principal limitations: limited reporting FVB/N-Tg.AC (v-Ha-*Ras*) transgene insert (single copy or hemizygous state); acetone control group treated with 200 µL/d; low-dose group was given 150 µL benzene 3×/wk; high-dose group was given 200 µL benzene 2×/d, 2×/wk

bw, body weight; d, day(s); F, female; h, hour(s); LOH, loss of heterozygosity; M, male; NOS, not otherwise specified; NR, not reported; NS, not significant; ppm, parts per million; wk, week(s)

3.3.1. Inhalation

Groups of 8 and 10 C57BL/6 wildtype and 8 and 13 h-Trx-Tg mice (overexpressing human thioredoxin) [sex and age at start not reported] were sham exposed (controls) or exposed by whole-body inhalation to benzene at a dose of 300 ppm [purity not reported], 6 hours per day, 5 days per week, for 26 weeks. Mice were maintained for their lifetime (or killed when showing symptoms of advanced haematopoietic neoplasms) and examined histopathologically. Survival of control h-Trx-Tg mice was higher than survival of the three other groups (which was comparable). There was a non-significant numerical increase in the cumulative incidence of lymphoma of the thymus gland (30%, 3/10) in the wildtype group exposed to benzene, while no lymphomas of the thymus gland were observed in the h-Trx-Tg group exposed to benzene (0/13) or in either of the control groups (Li et al., 2006). [The Working Group noted that this was a lifetime study, but the number of animals was small.]

Male B6.CBA-*Trp53*^{tm1Sia} congenic inbred mice (age, 8 weeks) (backcrossed repeatedly to C57BL/6 or B6 to homozygosity) were exposed to benzene at a concentration of 0, 33, 100, or 300 ppm [purity not reported, chemical grade] by whole-body inhalation for 6 hours per day, 5 days per week, for 26 weeks, and observed over their lifetime for tumour development (Kawasaki et al., 2009). Male B6.CBA-*Trp53*^{tm1Sia} mice heterozygous for a null and wildtype *Trp53* allele (haploinsufficient) were observed with a significant increase, with a significant positive trend, in the incidence of lymphoma of the thymus gland. [The Working Group noted that this was a lifetime study.]

Male C3.CBA-*Trp53*^{tm1Sia} congenic mice (age, 8 weeks) (backcrossed repeatedly to C3/He or C3 to homozygosity) were exposed to benzene at a concentration of 0, 100, or 300 ppm [purity not reported, chemical grade] for 6 hours per day, 5 days per week, for 26 weeks, and observed over their lifetime for the development of tumours (Kawasaki et al., 2009). Male C3.CBA-Trp53tm1Sia congenic mice heterozygous for a null and wildtype *Trp53* allele (haploinsufficient) were observed with significant increases in the incidence, with significant positive trends, of thymic lymphoma and of myeloid leukaemia, and a significant increase in the incidence of non-thymic lymphoma. [The Working Group noted that this was a lifetime study.]

3.3.2 Oral administration

Male B6.129-*Trp53*^{tm1Bra} N5 (the fifth C57BL/6 backcross generation, 97% homozygous) congenic mice (age, 7–10 weeks), heterozygous for a null and wildtype *Trp53* allele (Donehower et al., 1992), were given benzene [purity not reported] at a dose of 0 (vehicle only; $n = 20$) or 200 mg/kg bw ($n = 40$) by gavage (vehicle, corn oil) for 5 days per week, for 26 weeks (French & Saulnier, 2000; French et al., 2001b). After 26 weeks of exposure, the *Trp53* haploinsufficient mice were observed with sarcoma (of the subcutis, around the head and neck region or thoracic cavity) (0/20, 16/39 [$P \leq 0.004$, Fisher exact test]), lymphoma of the thymus (0/20, 3/39), and acinar cell carcinoma of the pancreas (0/20, 1/39), but without tumours of the lung (0/20, 0/22). Loss of the residual *Trp53* wildtype allele was observed in tested sarcoma (subcutis) (13/16) and lymphoma of the thymus (3/3).

Benzene [purity not reported] was used as a positive control in the Alternatives to Carcinogenicity Testing project of the International Life Sciences Health and Environmental Sciences Institute in two studies. Male and female B6.129-*Trp53*^{tm1Bra} N5 congenic mice (age at start not reported), heterozygous for *Trp53* wildtype and null allele (Donehower et al., 1992), were given benzene at a dose of 0 or 100 mg/kg bw by gavage, for 5 or 7 days per week, for 26 weeks (Storer et al., 2001). There were 15 males and 15 females per group per study.

Male *Trp53* haploinsufficient mice exposed to benzene showed a non-significant increase in the incidence of lymphoma of the thymus, supported by a significant increase in the incidence of atypical hyperplasia of the thymus [preneoplastic lesion]. The incidence of lymphoma of the thymus was 0/30 and 4/30 (males) and 1/30 and 1/30 (females), and of atypical hyperplasia of the thymus 0/30 and 7/30 [$P \leq 0.01$] (males) and 0/30 and 2/30 (females), for control and exposed groups, respectively. [The Working Group noted the combination of data from two different studies with a similar design.]

The National Toxicology Program tested the B6.129-*Cdkn2a*^{tm1Dep} congenic heterozygous mouse for a null and wildtype *Cdkn2a* allele with reduced expression of both the p16Ink4a and transcript variant p19Arf tumour-suppressor proteins (Serrano et al., 1996). Male and female mice (age, 7–8 weeks) were exposed to benzene (purity, > 99%) at a dose of 0, 25, 50, 100, or 200 mg/kg bw by gavage for 5 days a week, for 27 weeks. Malignant lymphoma was observed in males (0/15, 0/15, 0/15, 0/15, 5/15; $P = 0.021$, Fisher exact test [$P < 0.001$, Cochran–Armitage trend test]), but not in females. In addition, male *Cdkn2a* haploinsufficient mice showed several preneoplastic lesions (including bone marrow, thymus, and lymph node atrophy) associated with dose-related benzene exposure. Significantly increased preneoplastic lesions in female mice were restricted to lymph node (mesenteric) atrophy (NTP, 2007).

3.3.3 Skin application

The application of mutagenic or non-mutagenic chemicals to the shaved dorsal skin of the FVB/N-Tg.AC(v-Ha-*Ras*) mouse (Tg.AC for short) can result in squamous cell papilloma of the skin, which can convert to malignant skin neoplasms due to the abrogation of the first step in the two-step process of initiation–promotion of tumorigenesis (Leder et al., 1990).

A quantity of 200 µL of neat benzene [purity not reported] was applied to the skin of a group of 10–15 female Tg.AC mice twice a week, resulting in the rapid development of papillomas of the skin in 5 weeks. After 20 weeks, 76.9% [10/13] of treated Tg.AC mice had an average of 7.4 papillomas per mouse. A control group of 10–15 mice served as vehicle control [results not reported] (Tennant et al., 1995). [The Working Group noted that no results were given for controls. This study was inadequate for the evaluation of the carcinogenicity of benzene.]

Application of neat benzene [purity not reported] to the shaved dorsal skin of female Tg.AC mice (age, 7 weeks) at a dose of 0 (acetone control group), 400, 800, or 1600 µL per week for 20 weeks resulted in a significant increase in the incidence of squamous cell papilloma of the skin (3/5, 7/10, 8/10, 10/10 ($P < 0.05$)) and a significant increase in tumour multiplicity (1.4 ± 1.7, 7.0 ± 10.3, 10.6 ± 8.5 ($P < 0.05$), 12.6 ± 10.3 ($P < 0.05$)) (Spalding et al., 1999).

Compared with the above skin application studies, at lower benzene [purity not reported] exposure levels of 0 (acetone control group), 100, or 150 µL applied three times a week for 20 weeks in hemizygous Tg.AC mice (age, 8 weeks), the incidences of papilloma of the skin were reduced (males, 0/10, 0/10, 3/10; females, 0/10, 1/10, 1/10) after 26 weeks (Holden et al., 1998).

Blanchard et al. (1998) applied neat benzene [purity not reported] to the shaved dorsal skin of Tg.AC male and female mice (age at start not reported) in both hemizygous (single transgene copy) and homozygous (two copies of transgene) states at a dose of 0 (acetone control group) or 200 µL three times per week, for 20 weeks. A significant difference [$P < 0.05$] in the incidence of papilloma of the skin between hemizygous (males, 3/10; females, 4/10) and homozygous (males, 10/10; females, 9/10) Tg.AC mice was reported. [The Working Group noted that no results were given for controls. This study was inadequate for the evaluation of the carcinogenicity of benzene.]

Benzene [purity not reported] was applied neat to the shaved dorsal skin of homozygous Tg.AC female mice [age at start not reported] at a dose of 0 (acetone control group), 450, or 800 µL once per week, for 20 weeks. After an additional observation period of 12 weeks, the incidences of bone marrow leukaemia (granulocytic) (0/19, 4/14 ($P < 0.05$, Fisher exact test), 15/15 ($P < 0.05$, Fisher exact test)) were significantly increased (French & Saulnier, 2000). [The Working Group noted the limited reporting of the study.]

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