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

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Toxicokinetics*

(*a*) *Humans*

2-Butoxyethanol is well absorbed by ingestion, inhalation and through the skin.

Several cases of ingestion of 2-butoxyethanol from suicide attempts have been reported (see Table 16). Rambourg-Schepens *et al*. (1988) described a case report of a suicide attempt by ingestion of 250–500 mL of a window cleaner that contained 12% 2-butoxyethanol. The subject survived but had moderate haemoglobinuria on the 3rd day and peak urinary levels of butoxyacetic acid of approximately 40 g/g creatinine [estimated from a graph]. In another report that involved the ingestion of 8 oz (8 × 28.4 cm^3 = 227.2 mL) of a cleaner that contained 10–30% 2-butoxyethanol and 10–40% isopropanol, the peak blood concentration of butoxyacetic acid was 900 μg/L (6.8 μM) and declined with a half-life of 12.7 h in samples collected 100 min after ingestion and at different times over 3 days (McKinney *et al*., 2000). [This is longer than other reported half-lives and may be due to simultaneous treatment of the patient with ethanol to inhibit metabolism.] The subject survived with no reported haemolysis. A third case also involved ingestion of 360–480 mL of a window cleaner that contained 22% 2-butoxyethanol. The highest measured blood concentration of butoxyacetic acid was 4.86 mM in samples collected approximately 16 h after ingestion and at different times over 3 days (Gualtieri *et al*., 2003).

The blood-to-air partition coefficient of 2-butoxyethanol was reported to be 7965 (Johanson & Dynésius, 1988) and, as a consequence, it is well absorbed by inhalation. Kumagai *et al*. (1999) measured the concentration of 2-butoxyethanol in inspired and expired air of four volunteers exposed to 25 ppm [120.5 mg/m^3] for 10 min at rest and found that 80% of the 2-butoxyethanol was absorbed. Johanson *et al*. (1986a) exposed seven male volunteers by inhalation to 20 ppm [96.4 mg/m^3; 0.85 $mmol/m^3$] 2-butoxyethanol for 2 h during physical exercise. Respiratory uptake averaged 10.1 μmol/min or 57% of the amount inspired. The concentration of 2-butoxyethanol in blood reached a plateau of 7.4 μmol/L after 1–2 h and the apparent elimination half-life was 40 min. Jones and Cocker (2003) reported a similar apparent elimination half-life of 2-butoxyethanol from blood of 56 min (range, 41–84 min) after exposure of four volunteers to 50 ppm [241 mg/m^3] 2-butoxyethanol for 2 h at rest. Peak levels of the metabolite butoxyacetic acid were seen in blood 20 min after the 2-h exposure with a mean concentration of 35 μM (range, 28–43 μM) that declined with a half-life of 13 min (range, 2–42 min). Johanson *et al*. (1986a) reported that

Table 16. Toxic effects of 2-butoxyethanol after its ingestion by humans

Reference	Subject	Glass cleaner (% of 2-butoxyethanol)	Syndrome
Rambourg-Schepens *et al*. (1988)	50-year-old woman	250–500 mL (12%, ~30–60 g)	Deep coma, poor ventilation, metabolic acidosis, hypokalaemia, haemoglobinuria, oxoluria
Gijsenbergh *et al*. (1989)	23-year-old woman	~500 mL; estimated absorbed samples, 200–250 mL (12.7%, ~25–30 g)	Coma, hypotension, metabolic acidosis, anaemia, haematuria
Litovitz *et al*. (1991)	87-year-old woman	Unknown amount (6.5%)	Coma, hypotension, metabolic acidosis, hepatic and renal failure
Bauer *et al*. (1992)	53-year-old man	500 mL (9.1%, 45.5 g)	Coma, metabolic acidosis, non-cardiogenic pulmonary oedema
Nisse *et al*. (1998)	52-year-old woman	150 mL (9.1%, 13.65 g)	Poor ventilation, metabolic acidosis, renal injury
Burkhart & Donovan (1998)	19-year-old man	568–852 mL (25–35%, 113–255 g)	Neurotoxicity, lethargy, deep coma, hypotension, metabolic acidosis, aspiration pneumonitis
McKinney *et al*. (2000)	51-year-old woman	227 mL (10–30%, ~22.7–68 g)	Vomiting, lethargy, metabolic acidosis, hypotension
Osterhoudt (2002)	16-month-old girl	Unknown quantity (10–30%)	Mental state depression, metabolic acidosis
Gualtieri *et al*. (2003)	18-year-old man	360–480 mL (22%, ~80–105.6 g)	Central nervous system depression, metabolic acidosis

total blood clearance of 2-butoxyethanol was 1.2 L/min and that the steady-state volume of distribution was 54 L.

In-vivo studies have investigated the dermal absorption of 2-butoxyethanol liquid (Johanson *et al*., 1988; Jakasa *et al.*, 2004) and vapours (Johanson & Bowman, 1991; Corely *et al*., 1997; Jones *et al*., 2003). Johanson *et al*. (1988) conducted experiments of dermal exposure among five male volunteers; two or four of their fingers were exposed to neat 2-butoxyethanol for 2 h. Capillary blood samples were collected from the other arm and were analysed for 2-butoxyethanol. Urine was collected for 24 h and analysed for butoxyacetic acid. The percutaneous uptake rates of 2-butoxyethanol ranged from 7 to 96 nmol/min/cm^2. The authors estimated that, on average, 17% of the absorbed dose was excreted as butoxyacetic acid within 24 h. However, their method for determining butoxyacetic acid did not include hydrolysis of the glutamine conjugate and may therefore underestimate the total amount of butoxyacetic acid excreted. The percutaneous uptake rate ranged from 1 to 16 μmol/min when four fingers were exposed while the respiratory rate

ranged from 8 to 14 (average, 10) μmol/min when the same volunteers were exposed to 20 ppm [96.4 mg/m^3; 0.85 mmol/m^3] butoxyethanol vapour during light work (Johanson *et al.*, 1986a).

In a more recent study, six male volunteers were exposed by dermal application twice to 50%, once to 90% or once to undiluted (neat) 2-butoxyethanol for 4 h on a 40-cm^2 area of skin. Inhalation exposure with a known input rate and duration was used as a reference dose. Dermal absorption parameters were calculated from 24-h excretion of total (free plus conjugated) butoxyacetic acid in urine and 2-butoxyethanol in blood, measured after both inhalation and dermal exposures. These exposures correlated to pulmonary uptake and dermal uptake. The dermal absorption of 2-butoxyethanol was higher from the aqueous solutions than from neat 2-butoxyethanol. The dermal fluxes obtained from 24-h cumulative excretion of butoxyacetic acid were 1.34 ± 0.49, 0.92 ± 0.6 and 0.26 ± 0.17 mg/cm^2/h for 50%, 90% and neat 2-butoxyethanol, respectively. The permeation rates into the blood reached a plateau between 60 and 120 min after the start of exposure, which indicated the achievement of steady-state permeation. The apparent permeability coefficient was 1.75 ± 0.53 × 10^{-3} and 0.88 ± 0.42 × 10^{-3} cm/h for 50% and 90% 2-butoxyethanol, respectively. These results show that percutaneous absorption of 2-butoxyethanol increases markedly in aqueous solutions and that a water content as low as 10% can increase permeation rates fourfold. The uptake after dermal exposure to aqueous solutions substantially exceeds pulmonary uptake (Jakasa *et al.*, 2004).

In-vitro studies with human epidermal membranes in glass diffusion cells with water as a receptor fluid show steady-state absorption rates of 0.198 (± 0.7) mg/cm^2/h for neat 2-butoxyethanol applied as an infinite dose. The permeability constant was 2.14 × 10^4 cm/h and the lag time was less than 1 h (Dugard *et al*., 1984). Lower rates of absorption were seen with full thickness human skin and a tissue culture medium that contained 2% bovine serum albumin (used as receptor fluid), in which the steady-state flux was 544 ± 64 nmol/cm^2/h (0.046 mg/cm^2/h) (Wilkinson & Williams, 2002).

2-Butoxyethanol vapour is also well absorbed through the skin and can contribute significantly to the systemic dose. Corley *et al*. (1997) exposed one arm of each of six volunteers to 50 ppm [24.1 mg/m^3] 2-butoxyethanol vapour for 2 h. Blood was collected from both the exposed and unexposed arms and was analysed for 2-butoxyethanol and butoxyacetic acid. Urine was collected and analysed for butoxyacetic acid and its conjugates, ethylene glycol and glycolic acid. The concentration of 2-butoxyethanol was 1500 times greater in finger-prick blood from the exposed arm than that in venous blood from the unexposed arm. This confirmed that the previous estimates of 75% dermal absorption of 2-butoxyethanol calculated by Johanson and Bowman (1991) were probably overestimates due to contamination of the blood sample with 2-butoxyethanol during collection. Estimates of dermal absorption based on butoxyacetic acid and a physiologically based pharmacokinetic model showed that dermal absorption of 2-butoxyethanol probably contributed 15–27% of the total systemic dose. After dermal exposure of one arm to 50 ppm for 2 h, the peak 2-butoxyethanol concentration was 0.07 μM [8.26 μg/L] and occurred at the end of exposure. The half-life for 2-butoxyethanol in blood was 0.66 h. The peak blood concentration of butoxyacetic acid

was 0.59 μM [70 μg/L] and occurred 3.7 h after the end of exposure. The half-life of butoxyacetic acid in blood was 3.3 h.

The significance of dermal absorption of 2-butoxyethanol vapour was confirmed by Jones *et al*. (2003) who exposed four volunteers on nine occasions by either 'whole body' (inhalation and dermal absorption) or 'skin only' (breathing clean air) exposure to 50 ppm 2-butoxyethanol for 2 h at different temperatures and humidities. At 25 °C and 40% relative humidity, dermal absorption of vapour accounted for an average of 11% of the total absorbed dose. Dermal absorption increased slightly with increased temperature or humidity; when both were combined (30 °C and 60% relative humidity), dermal absorption contributed 39% of the 'total' absorbed dose.

In humans, the elimination of 2-butoxyethanol is mostly by excretion of butoxyacetic acid in the urine. Johanson *et al*. (1986a) showed that less than 0.03% of the dose was excreted as unchanged 2-butoxyethanol in urine and that butoxyacetic acid (without hydrolysis) accounted for 15–55% of the dose. This value probably underestimates the percentage of 2-butoxyethanol that is excreted as butoxyacetic acid since this metabolite is also excreted as a glutamine conjugate. Sakai *et al*. (1994) showed that the percentage of conjugation of butoxyacetic acid in the urine of workers exposed to 2-butoxyethanol varied from 44 to 92% with a mean value of 71%, a value supported by Corely *et al*. (1997) who estimated butoxyacetic acid–glutamine conjugation to be around 67% and found no ethylene glycol or glycolic acid in the urine. Jones and Cocker (2003) showed that conjugation of butoxyacetic acid was variable both between and within workers and, based on urine samples from 48 workers, that the average level of conjugation was 57% (95% CI, 44–70%).

Studies of volunteers showed that peak urinary excretion of butoxyacetic acid occurs 3–6 h after the end of a 2-h exposure to 2-butoxyethanol by inhalation and then declines with a half-life of approximately 6 h (Johanson *et al*., 1986a; Jones & Cocker, 2003).

The low renal clearance of butoxyacetic acid (23–39 mL/min) indicates extensive binding to protein and absence or low efficiency of tubular secretion of butoxyacetic acid. The low apparent volume of distribution (15 L) is an additional indication of binding of butoxyacetic acid to blood proteins (Johanson & Johnsson, 1991).

(*b*) *Animals*

(i) *In-vivo studies*

2-Butoxyethanol is well absorbed from the stomach in experimental animals.

Poet *et al*. (2003) administered 250 mg/kg bw 2-butoxyethanol by gavage and by intraperitoneal injection and 400 mg/kg bw by subcutaneous injection to B6C3F_1 mice and found no significant differences at the end of exposure in blood concentrations of 2-butoxyethanol or butoxyacetic acid between the routes of administration. 2-Butoxyethanol was rapidly eliminated and was no longer detectable 1 h after treatment. The highest measured concentrations of butoxyacetic acid in blood (approx. 1 mM) were found in the samples obtained 0.5 h after administration.

Medinsky *et al*. (1990) administered [^{14}C]2-butoxyethanol in the drinking-water (at concentrations of 290, 860 or 2590 ppm equivalent to 237, 401 or 1190 μmol/kg bw, respectively) for 24 h to Fischer 344N rats and showed that 50–60% of the dose was eliminated in the urine as butoxyacetic acid, 10% as ethylene glycol, 8–10% as carbon dioxide and less than 5% as unmetabolized 2-butoxyethanol.

Sabourin *et al.* (1992a) exposed Fischer 344 rats by inhalation for 6 h to [^{14}C]2-butoxyethanol at concentrations up to 438 ppm [2111 mg/m^3] (at which level 50% died). Uptake and metabolism were linear up to 438 ppm and the major metabolite was butoxyacetic acid with lesser amounts of ethylene glycol and its glucuronide. Over 80% of the [^{14}C]2-butoxyethanol-derived material in blood was in the plasma and the data indicated that formation of the haemolytic metabolite butoxyacetic acid was linearly related to the exposure concentration up to levels that were toxic. Elimination of inhaled 2-butoxyethanol is rapid with half-lives of < 10 min in Fischer 344 rats and < 5 min in B6C3F$_1$ mice after 1 day of exposure (Dill *et al*., 1998).

Johanson (1994) exposed Sprague-Dawley rats by inhalation continuously for up to 12 days to 20 or 100 ppm [96.4 or 482 mg/m^3] 2-butoxyethanol and showed that it was efficiently metabolized with an average blood clearance of 2.6 L/h/kg. The major (64%) metabolite was butoxyacetic acid and its renal clearance averaged 0.53 L/h/kg. The kinetics of the elimination of 2-butoxyethanol and butoxyacetic acid were linear up to 100 ppm. The average blood concentration of butoxyacetic acid during exposure to 20 and 100 ppm was 41 and 179 μM, respectively.

After topical application of 200 mg/kg bw [^{14}C]2-butoxyethanol to an area of 12 cm^2 of the shaved backs of Wistar rats under non-occlusive conditions, 25–29% was absorbed within 48 h. Peak blood levels of 2-butoxyethanol occurred 2 h after application and butoxyacetic acid was the major metabolite. Haemolysis was noted in rats that received a single dermal application of 260–500 mg/kg bw. In-vitro studies of percutaneous penetration of 2-butoxyethanol showed that, in fresh dorsal skin of hairless rats under non-occlusive conditions, 6% of the dose was absorbed within 1 h (2-butoxyethanol was absorbed or evaporated after 1 h) and that a greater percentage (10%) was absorbed from a 10% aqueous solution of 2-butoxyethanol (Bartnik *et al*., 1987).

The absorption of 2-butoxyethanol through the skin of anaesthetized guinea-pigs was studied using one or two sealed glass rings (3.14 cm^2 each) that contained 1 mL diluted or undiluted 2-butoxyethanol on the clipped back of the animals. During the latter half of a 2-h exposure to undiluted 2-butoxyethanol of an area of 6.28 cm^2 of skin, the concentration of 2-butoxyethanol in blood appeared to level off with an average concentration of 21 μmol/L (SD, 45%) and the absorption rate through the skin was estimated to be 0.25 (range, 0.05–0.46) μmol/min/cm^2 (SD, 49%) (Johanson & Fernström, 1986). In a later experiment, the relative rates of absorption of aqueous solutions were investigated; 5, 10 and 20% 2-butoxyethanol had rates of absorption similar to undiluted 2-butoxyethanol but 40% and 80% solutions had double the rate. The permeability coefficient of guinea-pig skin for undiluted 2-butoxyethanol was 0.4×10^{-3} cm/h and that for 5% aqueous 2-butoxyethanol was 12×10^{-3} cm/h (Johanson & Fernström, 1988).

Three different amounts (520–2530 μmol/kg bw [30–61.4 mg/kg bw]) of [^{14}C]2-butoxyethanol were applied to three circular areas, 2 cm in diameter, of the clipped backs of Fischer 344N rats (non-occluded); within the dose range studied, absorption and metabolism were linear with dose and 20–25% of the dose was absorbed. The majority (83%) of the absorbed dose was excreted in the urine as butoxyacetic acid; only small amounts of ethylene glycol were formed. Eighty per cent of [^{14}C]2-butoxyethanol was associated with plasma and less than 20% was associated with red cells. Peak levels of [^{14}C]2-butoxyethanol (223 nmol/mL [^{14}C]2-butoxyethanol equivalents) in plasma were reached at about 1 h after dermal administration of 1530 μmol/kg bw [18.5 mg/kg bw] [^{14}C]2-butoxyethanol, after which the concentration decreased with a half-life of about 4 h. The major metabolite (53–75% of [^{14}C]2-butoxyethanol equivalents) in plasma was butoxyacetic acid (Sabourin *et al*., 1992b). Compared with other studies (Medinsky *et al*., 1990), the metabolic profile was slightly different after dermal application compared with administration in the drinking-water, which was speculated to be due to different rates of administration and/or local tissue metabolism (Sabourin *et al*., 1992b).

Johanson and Fernström (1986) gave an intravenous bolus dose of 42 or 92 μmol/kg bw (5 or 11 mg/kg bw) 2-butoxyethanol to pentobarbital-anaesthetized guinea-pigs. The apparent total clearance and mean residence time of 2-butoxyethanol were 128 mL/min/kg bw (± 30%, SD) (which corresponds to 2.7 mL/min/g liver (± 30%, SD)) and 4.7 min (± 30%, SD), respectively.

Ghanayem *et al*. (1990) injected [^{14}C]2-butoxyethanol intravenously into rats of different ages (controls aged 3–4 months and old rats aged 12–13 months). In addition, some rats were also pretreated with pyrazole or cyanamide, which are inhibitors of alcohol dehydrogenase (ADH) or aldehyde dehydrogenase (ALDH), respectively, or probenecid, which is an inhibitor of renal transport of organic acids. The area-under-the-curve (AUC), maximum blood concentration (C_{max}) and systemic clearance of 2-butoxyethanol were dose-dependent. There was no effect of dose on half-life or volume of distribution of 2-butoxyethanol. Age did not effect the half-life, volume of distribution or clearance of 2-butoxyethanol but C_{max} and the AUC of 2-butoxyethanol increased in older rats. Inhibition of ADH and ALDH, the enzymes that metabolize 2-butoxyethanol, increased the half-life and AUC of this substrate and decreased its clearance. For butoxyacetic acid, the half-life, AUC and C_{max} increased in older rats and with dose. Inhibition of ADH and ALDH decreased the C_{max}, AUC and half-life of butoxyacetic acid. Treatments that protect against 2-butoxyethanol-induced haemolytic anaemia in rats were associated with a significant decrease in the concentrations of butoxyacetic acid in blood. When renal transport of organic acids was inhibited, no effect on the AUC, C_{max} or clearance of 2-butoxyethanol but an increase in the half-life and AUC of butoxyacetic acid were observed. The data suggest that a decreased elimination of butoxyacetic acid in older rats may contribute to their increased sensitivity to 2-butoxyethanol-induced haematotoxicity.

Bartnik *et al*. (1987) injected Wistar rats subcutaneously with 118 mg/kg [^{14}C]2-butoxyethanol; 79% of the radioactivity was excreted in the urine, 10% in expired air (as carbon dioxide) and 0.5% in the faeces within 72 h. Thymus and spleen had higher levels

of radioactivity than liver, fat, kidney, testes, sternum, carcass and blood [other tissues not examined].

In female $B6C3F_1$ mice, 2-Butoxyethanol and butoxyacetic acid were eliminated more slowly from forestomach tissue than from the blood or other tissues. The half-lives of 2-butoxyethanol after an intraperitoneal dose of 261 mg/kg bw were 2.6 h and 0.6 h for the forestomach and liver, respectively. The same dose of 2-butoxyethanol resulted in half-lives of butoxyacetic acid of 4.6, 1.1 and 1.05 h for the forestomach, liver and blood, respectively. The forestomach was the only tissue that had detectable levels of 2-butoxyethanol at 24 h. 2-Butoxyethanol and butoxyacetic acid were excreted in the saliva and were present in the stomach contents for a prolonged period following intraperitoneal and oral administration (Poet *et al*., 2003).

Ghanayem *et al*. (1987a) administered 125 or 500 mg/kg bw [^{14}C]2-butoxyethanol to Fischer 344 rats by oral gavage and showed that 2-butoxyethanol was distributed at highest concentrations (at 48 h) in the forestomach followed by liver, kidney, spleen and glandular stomach. The tissue concentrations did not increase linearly from low to high doses. The major route of elimination was the urine followed by exhalation of [^{14}C]carbon dioxide. The proportion of the dose excreted as [^{14}C]carbon dioxide was significantly higher in rats treated with 125 mg/kg bw compared with those given 500 mg/kg bw, which may indicate saturation of metabolism. A small proportion of the dose was excreted in the bile (8% of 500 mg/kg bw). The major urinary metabolite was butoxyacetic acid which accounted for more than 75% of ^{14}C in the urine, and the second major metabolite was the glucuronide conjugate of 2-butoxyethanol. Conversely, the major metabolite in bile was the glucuronide conjugate followed by butoxyacetic acid. A small quantity of radioactivity was excreted in the urine as the sulfate conjugate of butoxyethanol at the lower (but not the higher) dose.

Species and sex differences in elimination exist and, overall, mice eliminated 2-butoxyethanol and butoxyacetic acid faster than rats. Sex-related differences were most significant in rats and females were less efficient at clearing butoxyacetic acid from blood than males. It was speculated that this might be explained by differences in renal excretion (Dill *et al*., 1998). As the animals aged, the rates of elimination of butoxyethanol and butoxyacetic acid decreased in both species. Old mice eliminated butoxyacetic acid from blood up to 10 times more slowly than young mice after a single exposure but this difference was reduced after repeated exposure of the old mice to 2-butoxyethanol.

Further evidence of the influence of ethanol on the elimination of 2-butoxyethanol was reported in an earlier study (Romer *et al*., 1985). Ethanol (20 mmol/kg bw [920 mg/kg bw]) and 2-butoxyethanol (2.5 mmol/kg bw [295 mg/kg bw]) were co-administered intraperitoneally to female Sprague-Dawley rats; blood levels of 2-butoxyethanol were nearly constant as long as blood ethanol levels were above 3 mM [138 µg/mL]. This level of ethanol inhibited the metabolism of 2-butoxyethanol by ADH, the enzyme that is common to ethanol and 2-butoxyethanol.

(ii) *In-vitro studies*

In-vitro studies with isolated perfused rat liver showed dose-dependent Michaelis-Menten kinetics in the elimination of 2-butoxyethanol at doses up to 3 mM [354 µg/mL] (Johanson *et al.*, 1986b). The apparent Michaelis-Menten constant (K_m) ranged from 0.32 to 0.7 mM [38 to 82.6 µg/mL] and the maximum elimination (V_{max}) rate ranged from 0.63 to 1.4 µmol/min/g liver [74.5–165 µg/min/g liver]. The maximum intrinsic clearance was 1.7–2 mL/min/g liver. This in-vitro study also investigated the influence of ethanol on the elimination of 2-butoxyethanol and found that, in the presence of 17 mM [782 µg/mL] ethanol, the extraction ratio of 2-butoxyethanol decreased from 0.44 to 0.11, which supports the hypothesis that 2-butoxyethanol is metabolized by ADH.

(iii) *Pharmacokinetic models*

Several pharmacologically based pharmacokinetic models have been developed to describe the absorption and elimination of 2-butoxyethanol in humans (Johanson & Näslund, 1988; Corley *et al*., 1997), rats (Shyr *et al*., 1993; Lee *et al*., 1998) or both (Johanson, 1986; Corley *et al*., 1994). The first model by Johanson (1986) was based on elimination data (K_m and V_{max}) that were extrapolated from perfused rat liver and used blood flows and tissue volumes from the literature. Simulation of arterial blood concentrations of 2-butoxyethanol in a pharmacologically based pharmacokinetic model developed for a man after inhalation exposure to 20 ppm [0.8 mmol/m^3] and physical exercise ('light work'; 50 W) agreed well with data from a study of experimental exposure of human volunteers. In further simulations, the effects of exercise and co-exposure to ethanol were also studied and both predicted increased blood concentrations of 2-butoxyethanol due to increased ventilation (e.g. increased pulmonary uptake of 2-butoxyethanol) and inhibition of its metabolism, respectively. The relatively rapid decay of 2-butoxyethanol in all compartments indicates that it is unlikely to accumulate. The model also predicted that linear kinetics can be expected following occupational inhalation exposures to 2-butoxyethanol of < 100 ppm.

Corley *et al*. (1994) developed a more sophisticated model to include additional routes of exposure, physiological parameters and competing metabolic pathways for 2-butoxyethanol. Model simulations were compared with data from rats following either intravenous infusion or oral or inhalation exposure and from humans following either inhalation or dermal exposure to 2-butoxyethanol. The model accurately simulated observed data and was used to show that the species differences in kinetics resulted in higher blood concentrations of butoxyacetic acid in rats than in humans. This, coupled with the fact that human blood is less susceptible to haemolysis by butoxyacetic acid, predicts less risk for haemolysis in humans as a consequence of exposure to 2-butoxyethanol. In a later model, Corley *et al*. (1997) added parameters to describe the dermal absorption of 2-butoxyethanol vapours and showed that, after exposure to 25 ppm (0.8 mmol/m^3) for 8 h, the concentrations of butoxyacetic acid in human blood would be unlikely to reach levels associated with haemolysis *in vitro*.

4.1.2 *Metabolism*

The general metabolism of 2-butoxyethanol is described in Figure 1 (ATSDR, 1998). In the primary pathway, which occurs in the liver, 2-butoxyethanol is first oxidized via ADH to the intermediate, 2-butoxyacetaldehyde, which is subsequently further oxidized via ALDH to 2-butoxyacetic acid (the principal active metabolite). 2-Butoxyacetic acid may be conjugated with glycine or glutamine to form *N*-butoxyacetylglycine and *N*-butoxyacetylglutamine, respectively, or be metabolized to carbon dioxide. 2-Butoxyethanol can also be *O*-dealkylated via cytochrome P450 (CYP) 2E1 to form ethylene glycol and butyraldehyde. Ethylene glycol is subsequently metabolized to oxalic acid and further to carbon dioxide, while butyraldehyde is oxidized to butyric acid. 2-Butoxyethanol can also be conjugated directly with glucuronide or sulfate via glucuronyl or sulfotransferases, respectively. In addition, conjugation with fatty acids in the liver has been observed in one in-vivo study in rats exposed to 2-butoxyethanol (Kaphalia *et al*., 1996).

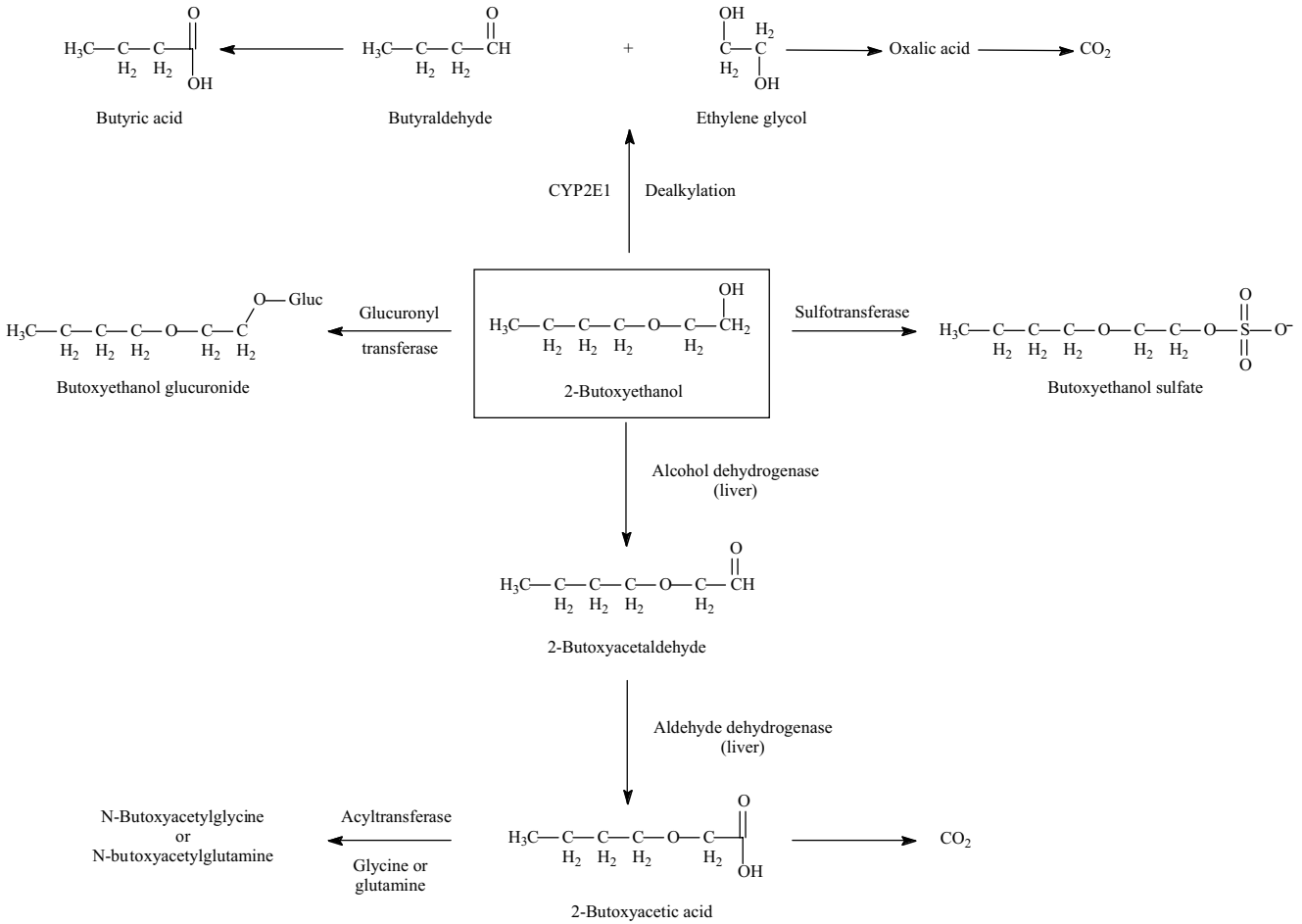
The route of administration appears to influence the relative importance of each metabolic pathway in rats, based on the profile of urinary metabolites (Medinsky *et al*., 1990; Sabourin *et al*., 1992a,b). Although 2-butoxyacetic acid is the major metabolite following exposure to 2-butoxyethanol via any route, the formation of ethylene glycol is favoured after inhalation exposure or administration in the drinking-water compared with conjugation with glucuronide. However, at low concentrations in the drinking-water or high concentrations in air, no significant difference in the proportion of these metabolites was observed. Production of the glucuronide conjugate is favoured following dermal exposure.

(*a*) *Oxidation of 2-butoxyethanol to 2-butoxyacetaldehyde and 2-butoxyacetic acid*

Although it was considered for a long time to be an intermediate step in the conversion of 2-butoxyethanol to butoxyacetic acid, the actual formation of the aldehyde metabolite has only very recently been demonstrated in experimental animals. Deisinger and Boatman (2004) administered a single oral dose of 600 mg/kg bw 2-butoxyethanol to male and female B6C3F_1 mice and detected low but measurable levels of 2-butoxyacetaldehyde in the blood, liver and forestomach at all time-points (5, 15 and 45 min) up to 90 min after exposure. Concentrations were highest in all tissues 5 min after administration and declined thereafter. Initial levels were approximately 10-fold higher in the forestomach than in the blood and liver. Levels of the aldehyde intermediate were significantly higher in tissues of female mice than in those of male mice, and were one to three orders of magnitude less than those of the acid metabolite.

An extensive database of studies in exposed humans and experimental animals shows that the formation of 2-butoxyacetic acid is a principal metabolic end-point for 2-butoxyethanol. Although it mainly occurs in the liver, there is potential for the formation of 2-butoxyacetic acid in tissues at or near the site of contact that contain ALDH and ADH, e.g. the skin, forestomach or glandular stomach (Sabourin *et al*., 1992a). Aasmoe *et al*. (1998) demonstrated that ADH3 was the only ADH isoenzyme that effectively oxidized

Figure 1. Metabolism of 2-butoxyethanol



Modified from ATSDR (1998)

2-butoxyethanol in the liver of rats. No information was found on the isoenzyme(s) of ADH that is involved in the metabolism of 2-butoxyethanol to 2-butoxyacetic acid in humans. There are several classes of ADH, for some of which interindividual variation occurs in humans (i.e. polymorphism); thus, there may be substantial variability in the extent to which 2-butoxyethanol is metabolized to 2-butoxyacetic acid in the human population, but this has not been investigated extensively.

Green *et al*. (2002) compared the distribution of ADHs in the forestomach and glandular stomach of rodents with that in the stomach of humans. The authors noted that the enzymes are largely concentrated in the stratified squamous epithelium of the forestomach of rats and mice but are more diffuse in the glandular stomach, and have greater activity in these tissues in mice than in rats. This greater activity corresponds to the greater sensitivity of mice to 2-butoxyethanol-induced effects in the forestomach. In the human stomach, ADH and ALDH are evenly distributed throughout the gastric epithelial cells of the mucosa.

2-Butoxyacetic acid appeared in the urine or was measured in the blood of humans following incidental ingestion or occupational or controlled exposure via inhalation or dermal contact (Johanson *et al*., 1986a, 1988; Rambourg-Schepens *et al*., 1988; Angerer *et al*., 1990; Rettenmeier *et al*., 1993; Sakai *et al*., 1993; Söhnlein *et al*., 1993; Sakai *et al*., 1994; Corley *et al*., 1997; Haufroid *et al*., 1997; Laitinen, 1998; McKinney *et al*., 2000; Jakasa *et al*., 2004). In these studies, 2-butoxyacetic acid was the major metabolite identified and was found to correspond to up to 70% of the amount of 2-butoxyethanol absorbed (Jakasa *et al*., 2004). Some of the 2-butoxyacetic acid is eliminated as the free acid, while a portion is conjugated before elimination (Jakasa *et al*., 2004). Concentrations of free 2-butoxyacetic acid in the blood of five men exposed to 20 ppm [97 mg/m^3] 2-butoxyethanol by inhalation for 2 h ranged from 18.5 to 56.5 μM [2 to 7 mg/L] (Johanson & Johnsson, 1991). Levels of 2-butoxyacetic acid at the end of a shift in the urine of workers who used glass cleaners that contained 2-butoxyethanol increased linearly with ambient concentration of 2-butoxyethanol (exposure range, < 0.1–7.33 ppm [< 0.5–35 mg/m^3]) (Vincent *et al*., 1993), which suggests that saturation of this metabolic pathway does not occur at these exposure concentrations. However, in an experimental study, Johanson *et al*. (1986a, 1988) observed substantial variation in the urinary levels of 2-butoxyacetic acid between volunteers exposed to 2-butoxyethanol by dermal application (2.5–39%; five subjects) or by inhalation (15–55%; six subjects). Similarly, Jakasa *et al*. (2004) observed interindividual variations of 42–70% in urinary levels of total 2-butoxyacetic acid (i.e. free and conjugated) of six volunteers exposed by inhalation to approximately 93 mg/m^3 2-butoxyethanol.

2-Butoxyacetic acid is also the major metabolite of 2-butoxyethanol in experimental animals. In rats administered up to 2590 ppm [2.6 mg/L] 2-butoxyethanol in the drinking-water (1.2 mmol/kg bw), 2-butoxyacetic acid eliminated in the urine accounted for up to 60% of the administered dose (Medinsky *et al*., 1990). 2-Butoxyacetic acid also comprised more than 75% of the radioactivity in the urine of rats administered a single dose of up to 500 mg/kg bw [^{14}C]2-butoxyethanol by gavage (Ghanayem *et al*., 1987a). Inhibition of

ADH or ALDH by pyrazole or cyanamide, respectively, significantly reduced the extent of conversion of 2-butoxyethanol to 2-butoxyacetic acid in rats administered a single oral dose of 500 mg/kg bw (i.e. from 75–90% of total radioactivity in the urine to 5–13%); the reduction in levels of 2-butoxyacetic acid corresponded with a reduction in toxicity (Ghanayem *et al*., 1987b) (see also Section 4.1.1). Similarly, competitive inhibition of ADH by ethanol, *n*-propanol or *n*-butanol reduced the production of 2-butoxyacetic acid from 2-butoxyethanol by 43, 33 and 31%, respectively (consistent with the greater affinity of the enzyme for alcohols than for glycol ether), which was accompanied by a corresponding reduction in the toxicity of 2-butoxyethanol (Morel *et al*., 1996). Exposure of rats to airborne concentrations of up to 483 ppm [2333 mg/m^3] 2-butoxyethanol did not appear to exceed the saturation level of its conversion to 2-butoxyacetic acid, based on the increase in concentrations of this metabolite measured in the urine (Sabourin *et al*., 1992a; Johanson, 1994).

In an investigation of the metabolism of 2-butoxyethanol to 2-butoxyacetic acid during long-term inhalation exposure of rats and mice (Dill *et al*., 1998; National Toxicology Program, 2000), the rate of elimination of 2-butoxyethanol from blood followed linear kinetics, which suggests that the production of 2-butoxyacetic acid in both species was also linear. However, the rate of disappearance of 2-butoxyethanol from blood was greater in mice than in rats. Similarly, mice were more efficient at eliminating 2-butoxyacetic acid from the blood, although elimination followed non-linear kinetics in both species (see also Section 4.1.1(*b*)). In addition, the rate of elimination of both 2-butoxyethanol and 2-butoxyacetic acid from blood decreased with increasing duration of exposure, particularly in rats. Thus, although mice may metabolize 2-butoxyethanol to 2-butoxyacetic acid at a greater rate than rats, the metabolite is subsequently cleared much more rapidly in mice, which is consistent with the apparently greater sensitivity to 2-butoxyethanol-induced haematological effects of rats than mice in both short- and long-term studies. Similarly, the greater sensitivity of female rats than male rats is probably related to slower clearance of the active acid metabolite than to sex-related differences in its production, although the activity of the hepatic ADH isoenzyme involved (ADH3) was reported to be greater in female rats than in male rats (Aasmoe *et al*., 1998; Aasmoe & Aarbakke, 1999). The activities of gastric ADH are greater in male than in female rats [however, the sex difference was only significant when octanol was the substrate; 2-butoxyethanol was not tested in this study] (Aasmoe & Aarbakke, 1999). Consistent with the less pronounced sex-specific sensitivities to 2-butoxyethanol-induced toxicity in mice, little sex difference in the formation or elimination of 2-butoxyacetic acid was observed in this species.

Ghanayem *et al*. (1987c) observed that older rats metabolized 2-butoxyethanol to 2-butoxyacetic acid to a greater extent than younger rats following oral administration of 500 mg/kg bw, based on a comparison of the levels of 2-butoxyacetic acid in the urine. Similar results were obtained in rats that were administered a single intravenous dose of up to 125 mg/kg bw 2-butoxyethanol, based on greater values of the AUC and C_{max} of 2-butoxyethanol and in C_{max} and half-life of 2-butoxyacetic acid in older rats compared with younger rats (Ghanayem *et al*., 1990). In mice, although no significant difference in the rate of clearance of 2-butoxyethanol (and presumably the formation of 2-butoxyacetic

acid) was observed between older and younger animals exposed to up to 125 ppm [603 mg/m^3] by inhalation for 1 day, younger mice cleared 2-butoxyacetic acid at a 10-fold greater rate than older mice after 1 day. However, this difference was less obvious after 3 weeks of exposure (Dill *et al*., 1998) (see also Section 4.1.1(*b*)).

In an in-vitro investigation of the comparative metabolism of 2-butoxyethanol in hepatocytes from humans and rats, Green *et al*. (1996) observed that cells from rats were more efficient at converting 2-butoxyethanol to 2-butoxyacetic acid than those from humans (four men and three women). Saturation of this pathway appeared to occur at much lower doses in hepatocytes from humans than in those from rats, since the percentage of total radioactivity identified as 2-butoxyacetic acid was only 1.5-fold greater in rat hepatocytes than in human hepatocytes at 0.02 mM, while the difference was 6.1-fold at 10 mM. The percentage that was converted to 2-butoxyacetic acid increased with up to 10 mM glycol ether in rat hepatocytes but decreased in human hepatocytes between 0.02 and 0.2 mM.

(*b*) *Conjugation of 2-butoxyacetic acid with glutamine or glycine*

Conjugation of 2-butoxyacetic acid with glutamine via acyltransferase has been demonstrated in humans. Rettenmeier *et al*. (1993) reported that *N*-butoxyacetylglutamine accounted for a mean of 48% (range, 16–64%) of the 2-butoxyacetic acid that was detected in urine collected at the end of the work week from six lacquerers who were exposed to 2-butoxyethanol (the remainder was free 2-butoxyacetic acid). Hence, conjugation with glutamine may represent an important route of removal of 2-butoxyacetic acid. Sakai *et al*. (1994) reported that a larger proportion (mean, 71%; range, 44–92%) of total 2-butoxyacetic acid was present as conjugates [not further identified] in the urine of six workers who were exposed to 2-butoxyethanol. They also determined that the fraction of 2-butoxyacetic acid eliminated as conjugates decreased throughout the work week, which may reflect a decline in the capacity for conjugation with continued exposure. These data are consistent with the lower proportions of conjugated 2-butoxyacetic acid observed by Rettenmeier *et al*. (1993) in the urine of workers after several days of exposure. More recently, Jones and Cocker (2003) determined that the mean extent of conjugation of 2-butoxyacetic acid [not further identified] was 57% (95% CI, 44–70%) in a group of 48 exposed workers, in six of whom there was no evidence of conjugation. These authors also observed substantial intra-individual variability in volunteers who were exposed repeatedly to 50 ppm [242 mg/m^3] 2-butoxyethanol for 2 h (i.e. nearly 0–100%) and hypothesized that this large variability was not related to polymorphisms but to other factors such as levels of glutamine. Corley *et al*. (1997) observed that about two-thirds (67%) of the 2-butoxyacetic acid excreted in the urine of male volunteers exposed dermally (one arm) to airborne concentrations of 50 ppm [242 mg/m^3] 2-butoxyethanol was present as *N*-butoxyacetylglutamine.

Little information was available on the conjugation of 2-butoxyacetic acid with glycine. No evidence of conjugation with this amino acid was found in rats that were administered up to 2590 ppm [2590 μg/mL] 2-butoxyethanol in the drinking-water for 1 day and observed for 3 days after exposure (Medinsky *et al*., 1990) or in rats exposed

dermally to up to 2530 µmol/kg bw [298 mg/kg bw] (Sabourin *et al*., 1992b). However, glycine conjugation with the alkoxyacetic acid metabolite has been observed for other glycol ethers (2-methoxyethanol and 2-ethoxyethanol) (Jönsson *et al*., 1982; Cheever *et al*., 1984; Moss *et al*., 1985). Corley *et al*. (1994) did not observe conjugation of 2-butoxyacetic acid with amino acids in samples of blood or urine from rats that were administered single doses of up to 126 mg/kg bw by gavage.

On the basis of these data, it appears that clearance of 2-butoxyacetic acid via conjugation occurs to a greater extent in humans than in rats, although data on this metabolic step in rodents are sparse.

(*c*) *Dealkylation of 2-butoxyethanol to ethylene glycol and butyraldehyde*

Only limited data on the occurrence of *O*-dealkylation of 2-butoxyethanol to ethylene glycol and (presumably) butyraldehyde in humans are available. Ethylene glycol, or its metabolite (oxalate), have been detected in the plasma or urine from two individuals who had ingested cleaning products that contained 2-butoxyethanol (250–500 mL of a solution that contained 12% 2-butoxyethanol) (Rambourg-Schepens *et al.*, 1988) and 150 mL of a solution that contained 9.1% 2-butoxyethanol (Nisse *et al.*, 1998). Metabolic acidosis, which is commonly associated with ethylene glycol poisoning, was observed in both cases (Rambourg-Schepens *et al*., 1988; Nisse *et al*., 1998). Conversely, neither ethylene glycol nor its metabolites were detected in the serum or urine from two people who had ingested 2-butoxyethanol (200–250 mL of a product that contained 12.7% 2-butoxyethanol (~25–30 g) or 360–480 mL of a product that contained 22% 2-butoxyethanol (~80–105 g)) (Gijsenbergh *et al*., 1989; Gualtieri *et al*., 1995), although metabolic acidosis occurred in both cases. Similarly, Corley *et al.* (1997) did not detect ethylene glycol or its metabolic products in the urine of a group of volunteers who were exposed dermally to 50 ppm [242 mg/m^3] 2-butoxyethanol for 2 h.

There is stronger evidence for the occurrence of this metabolic pathway in rats. Medinsky *et al*. (1990) reported that ethylene glycol comprised 14–22% of total urinary radioactive metabolites in rats that were administered up to 2590 ppm [2590 µg/mL] [^{14}C]2-butoxyethanol in the drinking-water for 24 h. It also accounted for 2–11% of total ^{14}C in the urine of rats that were administered doses of 8.6 or 126 mg/kg bw by gavage (Corley *et al*., 1994), but was not detected in the urine of rats that were administered up to 500 mg/kg bw 2-butoxyethanol by gavage (Ghanayem *et al*., 1987a). [This discrepancy may have been due to the ^{14}C radiolabel being on the butoxy moiety instead of the ethanol moiety (National Toxicology Program, 2000).] Similarly, Sabourin *et al*. (1992b) identified 4.1–6.5% of urinary metabolites as ethylene glycol in rats that were exposed dermally to up to 2530 µmol/kg bw [298 mg/kg bw] 2-butoxyethanol. Contrary to the increase in the proportion of urinary ethylene glycol observed in rats exposed orally or dermally to increasing levels of 2-butoxyethanol (Medinsky *et al*., 1990; Sabourin *et al*., 1992b), the fraction of urinary metabolites identified as ethylene glycol decreased with increasing exposure level in rats exposed to airborne concentrations of 4.3, 49 and

438 ppm [21, 237 and 2119 mg/m^3] 2-butoxyethanol for 6 h (i.e. 16, 13.4 and 8%, respectively). The majority of ethylene glycol was excreted after cessation of exposure (Sabourin *et al*., 1992a).

In an in-vitro study, hepatocytes from both rats and humans (four men and three women) metabolized 2-butoxyethanol to ethylene glycol. However, ethylene glycol represented a greater proportion of total radioactivity in rat cells than in human cells, which indicates a possible greater propensity for this metabolic step in rats than in humans. In both rat and human hepatocytes, the percentage of 2-butoxyethanol that was metabolized to ethylene glycol decreased with increasing dose, which the authors suggested was indicative of saturation of this pathway at relatively low concentrations of 2-butoxyethanol (Green *et al*.,1996).

Haufroid *et al*. (1997) hypothesized that polymorphism for CYP2E1 may influence the extent to which 2-butoxyethanol may be dealkylated to ethylene glycol and butyraldehyde. This was based on the observation that the level of free 2-butoxyacetic acid in the urine of a worker who expressed the heterozygous c2 allele (c1/c2) did not change substantially throughout the workday, unlike the 30 other workers who expressed the homozygous c1 allele (c1/c1) in whom substantial increases in levels of urinary free 2-butoxyacetic acid occurred. The authors suggested that the lack of increase in urinary 2-butoxyacetic acid may be due to greater activity of the c2 allele compared with that of the c1 allele in *O*-dealkylation of 2-butoxyethanol as observed by Hayashi *et al.* (1991). It was noted that urine samples were not analysed for amino acid conjugates of 2-butoxyacetic acid, which could also have influenced the excretion of free 2-butoxyacetic acid. The genetic polymorphism that affects transcriptional regulation of human CYP2E1 could also contribute to interindividual differences in the metabolism of 2-butoxyethanol via the dealkylation pathway.

Although no information was available regarding the formation of butyraldehyde or the subsequent metabolic product, butyric acid, in humans or experimental animals, it is predicted that butyraldehyde is the other metabolite that would result from *O*-dealkylation of 2-butoxyethanol to ethylene glycol.

(*d*) *Glucuronide and sulfate conjugation of 2-butoxyethanol*

Information on the in-vivo formation of glucuronide or sulfate conjugates with 2-butoxyethanol was only available for rats, although the glucuronide conjugate has been tentatively identified in hepatocytes from both rats and humans exposed to 2-butoxyethanol *in vitro* (Green *et al*., 1996). In rats administered single oral doses of 125 or 500 mg/kg bw radiolabelled 2-butoxyethanol, the glucuronide conjugate represented up to 24% of total urinary metabolites. This conjugate was no longer detectable in the urine 48 h after administration of 125 mg/kg bw, while levels were similar at 8 and 48 h following ingestion of 500 mg/kg bw. The sulfate conjugate was detected in samples of urine taken 8 h after administration of 125 mg/kg bw but was not found in the urine from rats exposed to 500 mg/kg bw. The glucuronide conjugate was the major metabolite in bile and constituted up to 89% of biliary radioactivity 30 min after exposure; however, this

proportion declined to 54% after 8 h. The sulfate conjugate was not detected in the bile (Ghanayem *et al*., 1987a).

The glucuronide conjugate has also been identified as a minor metabolite in the urine of rats exposed to 290–2590 ppm [290–2590 µg/mL] [^{14}C]2-butoxyethanol in the drinking-water for 24 h (8–11% of total radioactive metabolites measured in urine) (Medinsky *et al*., 1990) and of rats administered 8.6 or 126 mg/kg bw [^{14}C]2-butoxyethanol by gavage (10–15% of total radioactive metabolites) (Corley *et al*., 1994). The proportion of urinary metabolites identified as 2-butoxyethanol–glucuronide increased with the level of exposure in rats exposed to airborne concentrations of 4.3–438 ppm [21–2119 mg/m^3] 2-butoxyethanol for 6 h (i.e. from 10.6 to 62.3%). The glucuronide conjugate is apparently cleared rapidly, since it constituted only 3.6–6.5% of urinary metabolites during the 7–16 h after cessation of exposure (Sabourin *et al*., 1992a). However, the proportion of urinary metabolites identified as the glucuronide conjugate did not increase in rats administered dermal doses of 520–2530 µmol/kg bw, but was similar at all doses (i.e. 13–15%) (Sabourin *et al*., 1992b).

Inhibition of the principal pathway of metabolism of 2-butoxyethanol (i.e. oxidation to 2-butoxyacetic acid) by injection of pyrazole or cyanamide (inhibitors of ADH and ALDH, respectively) into rats administered a single oral dose of 500 mg/kg bw [^{14}C]-2-butoxyethanol (labelled at C1) resulted in a shift to increased conjugation with glucuronide and sulfate. The proportions of these conjugates increased from 9–24% (glucuronide) and undetectable (sulfate) to 75–85% and 7.5–20%, respectively, of the total radioactivity in the urine. The shift towards the conjugation pathway corresponded with a reduction in 2-butoxyethanol-induced haematotoxicity (Ghanayem *et al.*, 1987b). Following intravenous administration of lower single doses of 2-butoxyethanol to rats (62.5 or 125 mg/kg bw), the glucuronide conjugate was only detected in the blood of animals that had also been administered pyrazole as an inhibitor of ADH (Ghanayem *et al*., 1990).

Ghanayem *et al*. (1987c) demonstrated that young rats produce a greater proportion of glucuronide conjugates and (as discussed above) proportionally less 2-butoxyacetic acid than older rats, which is consistent with the apparent lower sensitivity of young rats to the haematotoxic effects of 2-butoxyethanol. However, the sulfate conjugate was not detected in the urine of young rats.

4.2 Toxic effects

4.2.1 *Humans*

(*a*) *Oral exposure*

Several individual cases that involved consumption of a few hundred millilitres of glass cleaners that contained 6.5–35% 2-butoxyethanol have been reported (Gijsenbergh *et al.*, 1989; Rambourg-Schepens *et al.*, 1988; Litovitz *et al*., 1991; Bauer *et al.*, 1992; Burkhart & Donovan, 1998; Nisse *et al*., 1998; McKinney *et al*., 2000; Osterhoudt, 2002;

Gualtieri *et al.,* 2003). The main symptoms were hypotension, poor ventilation, coma, metabolic acidosis (probably due to the formation of butoxyacetic acid), renal impairment, haematuria and effects on the blood (erythropenia, reduced haematocrit and haemoglobin levels, haemoglobinuria and/or hypochromic anaemia) (Table 16). [Treatment by haemodialysis can lead to haemodilution that might account for a lower blood cell count but not for haemoglobinuria.] In a report of 24 cases of ingestion of up to 300 mL of glass-cleaning products that contained 0.5–10% 2-butoxyethanol that were included in a survey of childhood poisonings, all of the children were reported to be asymptomatic. Even at the highest estimated dose of 1850 mg/kg bw (300 mL of a glass cleaner that contained 8% 2-butoxyethanol (~24 g)) that was consumed by a 2-year-old child, no signs of metabolic acidosis or of haematological, renal or neurological adverse effects were manifest (Dean & Krenzelok, 1992).

(*b*) *Dermal exposure*

Percutaneous absorption of 2-butoxyethanol was investigated experimentally in five men, who kept two or four fingers immersed in 2-butoxyethanol for 2 h. None of the subjects exhibited an adverse reaction (Johanson *et al*., 1988). No evidence of strong adverse dermal effects or skin sensitization was observed in 201 individuals exposed dermally to 0.2 mL 10% 2-butoxyethanol in patches, although repeated applications did produce increasing erythema in several subjects (Greenspan *et al.*, 1995).

(*c*) *Inhalation exposure*

The effects reported by subjects who were exposed to 113 ppm [546 mg/m^3] 2-butoxyethanol for 4 h (two men), 195 ppm [942 mg/m^3] for two 4-h periods separated by a 30-min interval (the same two men and one woman) or 98 ppm [474 mg/m^3] for 8 h (two men and two women) included irritation to the eyes (probably due to direct contact with the vapours), nose and throat, a disturbance of taste, a slight increase in nasal mucous discharge and headache. In this study, the women appeared to be more sensitive to the induction of these effects than the men. In none of these trials was there any evidence of changes from pre-exposure values in erythrocyte fragility, blood pressure, pulse rate or urinary levels of glucose or albumin (Carpenter *et al.*, 1956).

In two studies in which groups of four or seven male volunteers were exposed by inhalation to 20 or 50 ppm [97–242 mg/m^3] 2-butoxyethanol for 2 h, no overt signs of toxicity were observed (Johanson *et al.*, 1986a; Johanson & Boman, 1991). In the earlier investigation, in which the intake of 2-butoxyethanol was estimated to be 2 mg/kg bw for the 2-h exposure at 20 ppm (during which time the men performed light physical exercise), no consistent effects on the lungs (ventilation or breathing rate) or the heart (electrocardiogram readings or heart rate) were observed (Johanson *et al.*, 1986a). In the subsequent study, the investigators distinguished between vapour inhalation (through the mouth alone) and dermal exposure to the vapour (with a mask to prevent inhalation); no toxic effects were evident following either exposure protocol (Johanson & Boman, 1991). In a more recent study, four volunteers exposed to 25 ppm [121 mg/m^3] 2-butoxyethanol

for 10 min also did not show overt signs of toxicity shortly after treatment (Kumagai *et al*., 1999).

Signs of respiratory or ocular irritation, headache, sore throat and a sore nose that would become raw and bleed were reported in small groups of workers exposed to 5 ppm [24 mg/m^3] or less 2-butoxyethanol vapours (Apol, 1986).

The effect of occupational exposure to 2-butoxyethanol on various haematological, renal and hepatic parameters was investigated in 31 male workers at a beverage production plant who were exposed to mean concentrations of 3.64 mg/m^3 2-butoxyethanol (range, 1.77–6.14 mg/m^3; 20 samples) or 2.20 mg/m^3 (range, 0.75–3.35 mg/m^3; 11 samples) for 1–6 years, based on limited exposure data. The workers were also exposed to unspecified concentrations of methyl ethyl ketone. These parameters were also measured in a comparison population of 21 unexposed workers, matched for sex, age and smoking habits. Blood samples were collected at the end of the shift, while urine samples were taken before and after the workshift. No differences were observed in erythrocyte count, reticulocyte count, haemoglobin concentration, mean corpusclar volume, mean corpuscular haemoglobin or erythrocyte osmotic resistance between exposed and unexposed workers. However, haematocrit was significantly lower ($p = 0.03$) in the exposed group, while their mean cell haemoglobin concentration was significantly higher ($p = 0.02$) than that of controls, although the absolute differences in mean values were small (i.e. 43.9 versus 45.5% and 336 versus 329 g/L, respectively) and the ranges of values overlapped. No correlation was found between any of the haematological parameters investigated and levels of butoxyacetic acid in the urine at the end of the workshift or the difference in levels of this acid before and after the workshift (used as parameter of internal exposure), and no differences were observed in the renal parameters investigated (serum creatinine or urinary retinol binding protein). With regard to hepatic parameters, no statistically significant differences in levels of aspartate aminotransferase (20.5 ± 4.9 versus 21.7 ± 6.3 IU/L) or alanine aminotransferase (20.5 ± 13.1 versus 28.6 ± 16.3 UI/L) in the plasma were found between exposed and unexposed workers. However, plasma concentration of alanine aminotransferase and levels of butoxyacetic acid in the urine were statistically significantly correlated ($r = 0.60$; $p = 0.0004$) (Haufroid *et al*., 1997).

Several hours after the floor in an unventilated office had been stripped using a solvent that contained 2-butoxyethanol and other substances, seven workers were exposed to airborne levels estimated to be 100–300 ppm (483–1449 mg/m^3) (based on symptoms) for 0.5–4 h. Subjects had immediate severe irritation of the eye and upper respiratory tract, nausea and presyncope which subsided within 3 days. [It was not specified in the brief account of these cases whether haematological parameters were examined immediately after exposure.] All but one of these workers reported recurrent upper respiratory irritation and the appearance of cherry angiomas 3 months later. Eight months after exposure, six of the seven workers had an increased blood cell sedimentation rate and mild hypertension, while skin lesions continued to appear and persisted. Follow-up for an additional 5 years in four of these workers revealed gradual disappearance of

most of these effects, with the exception of the cherry angiomas that continued to persist and develop (Raymond *et al*., 1998).

Collinot *et al*. (1996) investigated the potential of 2-butoxyethanol to induce hepatic enzymes in a group of 17 male foundry workers who were exposed to a maximum concentration of 7.5 ppm (36 mg/m^3) 2-butoxyethanol. Levels of *D*-glucaric acid (which is the end product of the glucuronic acid pathway and is an indicator of enzyme activity in the liver) in the urine of exposed workers were compared with those in 18 unexposed controls. Urinary excretion of *D*-glucaric acid was significantly greater in exposed subjects than in controls, although significant seasonal variability was also found among exposed workers (increase in *D*-glucaric acid of 165% in winter and 85% in summer when the doors were open and the 2-butoxyethanol concentration was lower). [The Working Group noted that many of these occupational studies involve mixed exposures, the effects of which cannot be ascribed specifically to 2-butoxyethanol.]

4.2.2 *Experimental systems*

(*a*) *In-vivo studies with single doses (acute effect)*

Based on LD_{50}s and LC_{50}s that range from 530 to 6700 mg/kg bw and from 450 to 700 ppm [2173–3381 mg/m^3], respectively, 2-butoxyethanol produces low to moderate toxicity in experimental animals following acute exposure (Smyth *et al*., 1941; Werner *et al*., 1943a; Carpenter *et al*., 1956; Smyth *et al*., 1962; Weil & Wright, 1967; Gingell *et al*., 1998). Haematological effects, as well as effects on the liver, kidney, lung and spleen, some of which may be secondary to haematotoxicity, have been observed in animals exposed acutely to lower doses or concentrations. For example, alterations in haematological parameters that are characteristic of haemolytic anaemia have been observed in rats administered single oral doses as low as 125 mg/kg bw, while haemoglobinuria was noted in older rats following administration of 32 mg/kg bw by gavage (Ghanayem *et al*., 1987c). Female rats were more sensitive to 2-butoxyethanol-induced haemolysis than male rats following administration of a single dose of 250 mg/kg bw by gavage, as the magnitude of changes in haematological parameters was greater in females than in males; in addition, the onset of haemolysis was faster in females than in males (Ghanayem *et al*., 2000). Exposure to airborne concentrations of 62 ppm [299 mg/m^3] for 4 h resulted in increased osmotic fragility of erythrocytes (Carpenter *et al*., 1956), whereas dermal exposure to 260 mg/kg bw for 6 h induced haemolysis in rats (Bartnik *et al*., 1987). Ghanayem *et al*. (1992) and Sivarao and Mehendale (1995) demonstrated that younger blood cells were more resilient to 2-butoxyethanol-induced haemolysis than older cells, as rats that had been bled several days before exposure to a single oral dose were less severely affected than rats that had not been bled.

2-Butoxyethanol is considered to be a mild-to-severe irritant to the skin and eyes of rabbits (range doses, 0.1–0.5 mL neat compound), and severity increased with duration of exposure (range, 1–72 h) (Carpenter & Smyth, 1946; Smyth *et al*., 1962; Kennah *et al*.,

1989; Jacobs, 1992; Zissu, 1995). There was no evidence of skin sensitization in one investigation in guinea-pigs (Zissu, 1995).

(*b*) *In-vivo studies with multiple exposures*

(i) *Short-term exposure*

Oral exposure

In short-term studies in rats exposed by the oral route, the blood was the principal target for the effects of 2-butoxyethanol. Haematological changes that are characteristic of haemolysis (including reductions in red blood cell count, haemoglobin levels and haematocrit values) have been reported by a number of investigators in rats exposed repeatedly to 2-butoxyethanol for 2–65 days (Grant *et al*., 1985; Krasavage, 1986; National Toxicology Program, 1989; Dieter *et al.*, 1990; Ghanayem *et al.*, 1992; Ezov *et al*., 2002). In most of the studies in which haematological parameters were measured, these changes were observed at all doses administered by gavage or in the drinking-water (i.e. 100 mg/kg bw per day). However, a no-observed-effect level (NOEL) of 30 mg/kg bw per day was reported in a study in rats exposed for only 3 days (which was designed primarily to investigate developmental toxicity) (National Toxicology Program, 1989). Ghanayem *et al.* (2001) observed an increase in the magnitude of haematological changes that were indicative of severe regenerative anaemia after repeated administration of 250 mg/kg bw 2-butoxyethanol per day by gavage for 1, 2 or 3 days to male and female Fischer 344 rats that were killed 24 or 48 h after the last dose; the onset of effects was faster in females. Thrombosis was observed in the lungs, nasal submucosa, eyes, liver, heart, bones and teeth, together with lesions that were consistent with acute infarction in the vertebrae and femur; again, onset was earlier in female rats than in males, which the authors hypothesized to be precipitated by initial stages of haemolysis induced by 2-butoxyethanol. Similarly, in male and female Fischer F344 rats administered two, three or four daily doses of 250 mg/kg bw 2-butoxyethanol by gavage and examined on days 2, 3, 4 and 29, Ezov *et al*. (2002) reported severe acute regenerative haemolytic anaemia, with faster onset in females, together with thrombosis and infarction in the heart, brain, lungs, eyes and bones mainly in female rats. In a separate report, it was noted that signs of intramedullary thrombosis in the femur and subsequent new bone growth were observed in rats examined on day 29 (Shabat *et al*., 2003). Morphological changes were noted in erythrocytes, the severity of which progressed with increasing exposures and was greater in females than in males. Red blood cells from these exposed rats were found to have increased adherence to an extracellular matrix derived from endothelial cells of the blood vessel wall; it was suggested by the authors that such increased adherence could lead to vascular occlusion and result in the observed thrombosis (Koshkaryev *et al*., 2003). Nyska *et al*. (1999a) also observed thrombosis of ocular blood vessels with retinal haemorrhage, necrosis and photoreceptor degeneration in female Fischer 344 rats administered 250 mg/kg bw 2-butoxyethanol per day by gavage for 3 consecutive days; thrombosis was also noted in the nasal cavity submucosa, teeth, femur and vertebrae. In

a subsequent investigation, Nyska *et al*. (2003) noted a correlation between thrombosis and expression of the vascular cell adhesion molecule, which included severe haemolysis, in the ocular vessels of female Fischer 344 rats administered three or four daily doses of 250 mg/kg bw 2-butoxyethanol. The authors suggested that this molecule functions in the pathogenesis of 2-butoxyethanol-related thrombosis by promoting adhesion of erythrocytes to the endothelium.

In some of the short-term studies in rats, effects on blood were observed to be reversible after cessation of exposure (Grant *et al*., 1985; Ghanayem *et al*., 1992). In a preliminary developmental toxicity study in which pregnant rats were exposed daily to 0, 150, 300 or 600 mg/kg bw 2-butoxyethanol on days 9–11 or 11–13 of gestation and then killed on day 20, severe haematological effects were observed in dams 24 h after exposure, although the severity of these effects decreased as time since exposure increased (National Toxicology Program, 1989). In addition, there was evidence that rats developed tolerance to or autoprotection against the haematological effects of 2-butoxyethanol, as the magnitude of the changes was smaller in pre-exposed rats (Ghanayem *et al*., 1992; Sivarao & Mehendale, 1995). However, an increase in the time between pre-treatment and challenge from 7 to 14 or 21 days resulted in higher mortality, and indicated that the autoprotective effect is gradually lost as the red blood cells age (Sivarao & Mehendale, 1995). Therefore, similar to the results of the acute toxicity studies, these data suggested to the authors that younger blood cells are more resilient to the induction of effects by repeated exposure to 2-butoxyethanol, and that the lethality of 2-butoxyethanol is related to its ability to induce haemolysis.

Other target organs in which 2-butoxyethanol induced effects in short-term oral studies in rats include the spleen, liver and kidneys. Relative weights of the liver and spleen were increased in pregnant rats animals exposed to 100 mg/kg bw per day or more for 3 days (National Toxicology Program, 1989) and in male rats administered 125 mg/kg bw per day for 12 days (Ghanayem *et al.*, 1992), while increased relative kidney weight was observed in pregnant rats exposed to 100 mg/kg bw per day or more for 3 days (National Toxicology Program, 1989). Ezov *et al*. (2002) noted that increases in absolute and relative spleen weights inversely correlated with decreases in red blood cell count in male and female rats administered 250 mg/kg bw 2-butoxyethanol daily for up to 4 days. Microscopic examination revealed congestion of the spleen in rats after 6 weeks of exposure to 222, 443 and 885 mg/kg bw per day, while haemosiderin deposition in the liver and kidneys and haemoglobinuria were noted after exposure to 443 mg/kg bw per day and above (Krasavage, 1986). In contrast, no effects on organ weights were observed in short-term studies in which rats were exposed to up to 506 mg/kg bw per day for up to 21 days (Exon *et al*., 1991; National Toxicology Program, 1993) or up to 225 mg/kg bw per day for as long as 65 days (Dieter *et al.*, 1990).

Data on the short-term toxicity of orally administered 2-butoxyethanol in mice are more limited. No specific target organs were identified in a limited 2-week range-finding study at doses of up to 627 and 1364 mg/kg bw per day (in males and females, respectively) in the drinking-water, although the relative thymus weight of the males was

decreased at 370 mg/kg bw per day and above (National Toxicology Program, 1993). Dehydration was reported in some animals that were exposed to the highest doses (370 and 627 mg/kg bw per day in males and 673 and 1364 mg/kg bw per day in females), although decreased drinking-water consumption was only observed in one of the groups that was reported to have symptoms of dehydration. A reduction in red blood cell count was observed in mice administered 500 or 1000 mg/kg bw per day on 5 days per week for 5 weeks, but haemoglobin levels were not affected (Nagano *et al.*, 1979, 1984).

In an investigation of the potential mode of action of the induction of forestomach tumours in mice, Poet *et al*. (2003) administered 2-butoxyethanol by gavage to male and female B6C3F$_1$ mice at doses of 0, 400, 800 or 1200 mg/kg bw per day for 2 days, which were reduced to 0, 200, 400 or 600 mg/kg bw per day for an additional 2 days. Epithelial hyperplasia and inflammation of the forestomach were observed at all doses, and severity increased with dose. Similar effects were also observed in mice administered 400 mg/kg bw per day by intraperitoneal or subcutaneous injection for 4 days. Green *et al*. (2002) observed hyperkeratosis of the forestomach in female B6C3F$_1$ mice administered 500 mg/kg bw per day 2-butoxyethanol for 10 days, but not in the forestomach of mice exposed to 150 mg/kg bw per day or less or in the glandular stomach in any dose group.

Inhalation exposure

In short-term inhalation studies in rats, the blood, liver and kidneys were the main targets of 2-butoxyethanol toxicity, although examination was limited to gross pathology (Dodd *et al.*, 1983) or to the microscopic appearance of a limited range of tissues (Carpenter *et al.*, 1956). Increased erythrocyte fragility was reported in an early study in rats after 30 exposures (7 h per day on 5 days per week for 6 weeks) to 54 ppm [261 mg/m^3] 2-butoxyethanol and above (Carpenter *et al.*, 1956). In Fischer 344 rats exposed to 0, 20, 86 or 245 ppm [0, 97, 415 or 1183 mg/m^3] for 9 days (6 h per day), haematological effects observed at the two higher concentrations included significantly decreased red blood cell count and haemoglobin concentration and increased reticulocyte count and mean corpuscular volume; with the exception of mean corpuscular volume, these changes were reversible during a 14-day recovery period. Increases in relative liver weight were observed at 85 ppm and above, and reversible changes in relative liver weight were also noted at 245 ppm and above. No effects were noted at 20 ppm (Dodd *et al*., 1983). In another earlier investigation, Werner *et al*. (1943b) observed decreases in red blood cell count and haemoglobin, accompanied by increases in reticulocyte count, in Wistar rats exposed to 135 or 320 ppm [653 or 1549 mg/m^3] 2-butoxyethanol for 5 weeks (7 h per day on 5 days per week).

In a study that was primarily designed to investigate developmental toxicity, pregnant Fischer 344 rats were exposed to 0, 25, 50, 100 or 200 ppm [0, 121, 242, 483 or 966 mg/m^3] 2-butoxyethanol for 10 days. The animals were killed on day 21 of gestation and haematological analyses were carried out on the dams. Similar to the effects reported by Dodd *et al*. (1983), significant decreases in red blood cell count and mean corpuscular

haemoglobin concentration and increases in mean corpuscular volume and mean corpuscular haemoglobin were observed in dams exposed to 100 ppm and 200 ppm; significant decreases in haemoglobin and haematocrit and increased relative spleen and relative kidney weights were noted after exposure to 200 ppm. No effects were observed after exposure to 50 and 25 ppm (Tyl *et al*., 1984).

In the only available short-term inhalation study in mice, a reversible increase in erythrocyte fragility was observed in animals exposed to 100 ppm [483 mg/m^3] for 30 exposures; transient haemoglobinuria was evident after exposure to 200 and 400 ppm [966 and 1932 mg/m^3] (Carpenter *et al.*, 1956).

Exposure of pregnant New Zealand white rabbits to up to 200 ppm [966 mg/m^3] 2-butoxyethanol for 13 days did not result in clear alterations in haematological parameters; however, there was a suggestion of haematuria/haemoglobinuria after exposure to high concentrations (100 and 200 ppm). In addition, mortality and the occurrence of spontaneous abortions were increased by 200 ppm, but not by 100 ppm or less (Tyl *et al*., 1984).

Limited data are also available on the short-term toxicity of 2-butoxyethanol in other experimental species. No haematological effects were noted in guinea-pigs exposed to up to 494 ppm [2386 mg/m^3] 2-butoxyethanol, although effects were noted in the lungs (congestion) and kidney (tubule swelling and increased relative weight) (Carpenter *et al*., 1956). However, effects on blood parameters (including increased leukocyte count, decreased erythrocyte count and haemoglobin and increased erythrocyte osmotic fragility) were noted in a small number of dogs exposed to 385 ppm [1860 mg/m^3] and in monkeys exposed to 200 or 100 ppm [966 or 483 mg/m^3] (Carpenter *et al*., 1956). Werner *et al*. (1943c) also observed slight, but statistically significant effects on haematological parameters (decreased haematocrit and haemoglobin) in dogs exposed to 415 ppm [2005 mg/m^3] for up to 12 weeks (7 h per day on 5 days per week).

Dermal exposure

Only limited data are available on the short-term dermal toxicity of 2-butoxyethanol. Based on a limited secondary account of an unpublished study (Tyler, 1984), haematological effects (reductions in red blood cell count and haemoglobin concentration) and local skin damage (erythema, oedema and necrosis) were reported in rabbits that received nine applications of an aqueous solution, with a lowest-observable-effect level (LOEL) of 180 mg/kg bw per day (applied as a 50% aqueous dilution) and a NOEL of 90 mg/kg bw per day (applied as a 25% aqueous dilution).

(ii) *Subchronic exposure*

Based on the limited database available, haematological effects and effects on the liver and kidneys appear to be the critical effects associated with subchronic exposure via ingestion or inhalation in animals.

Oral exposure

In a study conducted by the National Toxicology Program (1993), male and female Fischer 344/N rats were administered 0, 750, 1500, 3000, 4500 or 6000 ppm 2-butoxyethanol in the drinking-water (estimated equivalent doses, 0, 69, 129, 281, 367 or 452 mg/kg bw per day in males and 0, 82, 151, 304, 363 or 470 mg/kg bw per day in females based on drinking-water consumption) for 13 weeks. Haematological effects indicative of anaemia were observed in both males and females exposed to 750–6000 ppm (equivalent to 69–452 mg/kg bw per day in males and 82–470 mg/kg bw per day in females). Females were more sensitive to the haematological effects than males, as alterations in blood parameters (including red blood cell count, haemoglobin concentration, mean cell volume, mean cell haemoglobin and haematocrit) were reported at all doses as early as 1 week after initiation of exposure and were still present at 13 weeks. In males, signs of mild anaemia were only observed at doses of 1500 ppm (129 mg/kg bw per day) and above. The authors suggested from the spectrum of haematological effects that the anaemia was regenerative (as indicated by increased numbers of reticulocytes) and that haemolysis was brought about by cell swelling (increased mean cell volume). Effects were also observed in the liver, including increased relative weights (82 and 367 mg/kg bw per day in females and males, respectively) and histopathological changes (hepatocellular degeneration with 304 and 281 mg/kg bw per day in females and males, respectively, cytoplasmic alterations in all exposed groups and pigmentation with 151 and 452 mg/kg bw per day in females and males, respectively). Although the authors noted that cytoplasmic alterations may be related to the induction of enzymes that are associated with glucuronide and sulfate conjugation of 2-butoxyethanol, an increase in relative liver weight (commonly associated with enzyme induction) was observed at all exposure levels in females, while such increases were only noted in males at higher doses. Hepatic pigmentation was considered to be secondary to haematotoxicity (with similar sex-related differences in sensitivity), while degenerative changes were considered to be compound-related and may represent a primary toxicity of 2-butoxyethanol. The lowest concentration administered (i.e. 750 ppm, or 82 and 69 mg/kg bw per day in females and males for haematological and hepatic effects, respectively) was found to be the LOEL.

In male and female B6C3F$_1$ mice administered 0, 750, 1500, 3000, 4500 or 6000 ppm 2-butoxyethanol in the drinking-water for 13 weeks (estimated equivalent doses, 0, 118, 223, 553, 676 or 694 mg/kg bw per day in males and 0, 185, 370, 676, 861 or 1306 mg/kg bw per day in females based on drinking-water consumption), the only effects observed in mice exposed to up to 6000 ppm (equivalent to 694 and 1306 mg/kg bw per day in males and females, respectively) were slight reductions in body-weight gain (6000 ppm in both sexes) and increased relative kidney weight in females at all exposure levels (i.e. 750 ppm or 185 mg/kg bw per day and above). However, the effects on kidney weight were considered to be secondary to reduced body-weight gain, as no histopathological changes were noted at the highest dose (National Toxicology Program, 1993). [However, the Working Group noted that relative kidney weights were increased at doses lower than

those associated with significant reductions in body-weight gain.] In contrast, however, Heindel *et al*. (1990) reported increased mortality in female Swiss CD-1 mice administered drinking-water that contained 1 or 2% 2-butoxyethanol (equivalent doses, 1300 and 2100 mg/kg bw per day) for 15 weeks. Similarly exposed males lost weight during the study. No such effects were observed at doses of 700 mg/kg bw per day. No lesions of the kidney were observed in females exposed to 1300 mg/kg bw per day [the only dose at which examinations appear to have been conducted]. At this dose, relative kidney weights were increased in both sexes and relative liver weight was increased in the females. [It is unclear whether organ weights were examined at other doses.] The NOEL, based on changes in body-weight gain and organ weights, was considered to be 700 mg/kg bw per day; however, it is not indicated whether organ weights were examined at this dose. Haematological parameters were not investigated in either of these two studies.

In a study designed to investigate potential modes of induction of the liver neoplasms observed in a previously reported chronic bioassay in mice (National Toxicology Program, 2000), Siesky *et al*. (2002) administered 2-butoxyethanol by gavage to male $B6C3F_1$ mice and male Fischer 344 rats at doses of 0, 225, 450 and 900 mg/kg bw per day (mice) and 0, 225 and 450 mg/kg bw per day (rats) for up to 90 days; animals were killed after 7, 14, 28 and 90 days of exposure. Dose-related decreases in haematocrit and increases in relative spleen weight were observed in both species, accompanied by an increase in iron deposition in Kupffer cells. Oxidative DNA and lipid damage, as measured by 8-hydroxydeoxyguanosine and malondialdehyde, respectively, and increased DNA synthesis in hepatocytes and endothelial cells were observed in mice after 7 and 90 days of exposure to 2-butoxyethanol, but not in rats at any time point. Park *et al.* (2002a) observed similar indications of oxidative stress in $B6C3F_1$ mice exposed to 450 or 900 mg/kg bw 2-butoxyethanol per day by gavage for 7 days, together with decreased levels of the antioxidant, vitamin E. In an accompanying in-vitro study, these authors induced oxidative stress in isolated mouse hepatocytes exposed to haemolysed red blood cells, but not in hepatocytes exposed to 2-butoxyethanol or 2-butoxyacetic acid, which, they suggested, was indicative that the oxidative stress in the liver in mice was secondary to the accumulation of iron that resulted from 2-butoxyethanol-induced haemolysis. Based on the results of these studies, Park *et al*. (2002a) hypothesized that the liver tumours in mice arise due to stress associated with iron deposition in the liver, although, as noted below, the incidence of hepatic haemosiderin pigmentation did not correlate with the presence of tumours (National Toxicology Program, 2000) (see Section 4.5).

Inhalation exposure

In Fischer 344/N rats exposed to 0, 31, 62.5, 125, 250 or 500 ppm [0, 150, 302, 604, 1208 or 2415 mg/m^3] 2-butoxyethanol by inhalation for 14 weeks (6 h per day on 5 days per week), changes in haematological parameters that were characteristic of macrocytic, normochromic, responsive anaemia (i.e. increased mean cell volume, lack of change in mean cell haemoglobin values and increased reticulocyte count) were observed (National

Toxicology Program, 2000). Females were more sensitive than males; alterations in haematological parameters were observed at the lowest concentration tested (i.e. LOEL, 31 ppm [150 mg/m^3]) in females, while the LOEL in males for these effects was 125 ppm [604 mg/m^3]; the NOEL in males was found to be 62.5 ppm [302 mg/m^3]. The severity of these effects increased with concentration in both sexes. In addition, females that died or were killed before the end of the study had an increased incidence of thrombosis in the blood vessels of several tissues as well as bone infarction. Signs of earlier infarction were also observed in the vertebrae of females exposed to 500 ppm and killed at the end of the study period, although there was no evidence of thrombosis in these animals. These effects were not observed in male rats. It was hypothesized by the authors that the thrombosis resulted from severe acute haemolysis, which caused a release of protocoagulants from erythrocytes, or from anoxic damage to endothelial cells; either of these effects could compromise blood flow (see also Nyska *et al*., 1999b). Long *et al*. (2000) also reported thrombosis and infarction of the dental pulp of female rats that were exposed to 500 ppm [2415 mg/m^3] for 13 weeks and killed early (day 4 of the treatment) due to their moribund condition, but not in rats that were killed at termination of the study; they suggested that this was indicative of reversibility of the lesions. Other effects consistent with regenerative haemolytic anaemia observed in both male and female rats included excessive haematopoietic cell proliferation in the spleen, haemosiderin pigmentation in hepatic Kupffer cells and renal cortical tubules and bone marrow hyperplasia. Inflammation and/or hyperplasia of the forestomach also occurred in male and female rats exposed to the higher concentrations (250 and 500 ppm [1208 and 2415 mg/m^3]), while increases in relative kidney and liver weights were noted in females exposed to 62.5 ppm [302 mg/m^3] and above and in males exposed to 250 ppm and above (National Toxicology Program, 2000).

Haematological effects that consisted of slight decreases in red blood cell count and haemoglobin levels and an increase in mean corpuscular haemoglobin were also observed in female Fischer 344 rats exposed to 77 ppm [372 mg/m^3] (6 h per day on 5 days per week) for 6 weeks (Dodd *et al*., 1983); after 13 weeks of exposure, values for these parameters were generally similar to those of controls [contrary to the observations in this strain of rats by the National Toxicology Program (2000)]. Males appeared to be much less sensitive, as the only effect on blood was a very slight decrease in red blood cell count after 13 weeks of exposure to 77 ppm. No indication of haematotoxicity was observed at doses of 25 and 5 ppm [121 and 24 mg/m^3]. No histopathological changes or alterations in clinical chemistry were noted in exposed rats (Dodd *et al*., 1983).

Alterations in haematological parameters that were indicative of haemolytic anaemia (haemoglobin, haematocrit and erythrocyte counts) were also the most sensitive endpoints observed in B6C3F$_1$ mice exposed to 0, 31, 62.5, 125, 250 or 500 ppm [0, 150, 302, 604, 1208 or 2415 mg/m^3] (6 h per day on 5 days per week) for 14 weeks (National Toxicology Program, 2000). However, the anaemia in mice was considered to be normocytic, normochromic and responsive (compared with the macrocytic anaemia noted in rats), because 2-butoxyethanol did not induce any changes in mean cell volume. In addition, based on the magnitude of the changes, the anaemia was less severe in mice than in rats,

although females were again more sensitive than males (LOELs in females and males, 31 ppm [150 mg/m^3] and 125 ppm [604 mg/m^3], respectively). As in rats, effects in the spleen that were consistent with regenerative anaemia (haemosiderin pigmentation at doses of 125 ppm and above in males and 250 ppm and above in females and increased haematopoiesis at doses of 250 ppm and above in males and at 500 ppm in females) were also observed. The incidence of hyperplasia of the forestomach was increased in female mice exposed to 125 ppm or more, while the incidence of forestomach inflammation was increased in females exposed to 250 ppm and above. In males, only non-statistically significant increases in the incidence of forestomach hyperplasia were observed after exposure to the highest concentration. The incidence of haemosiderin pigmentation of hepatic Kupffer cells was increased in male mice at doses of 500 ppm and in female mice at doses of 250 ppm or above; haemosiderin pigmentation was also observed in the renal tubules of both males and females exposed to the highest concentration. Increased mortality was noted in both males and females exposed to 500 ppm.

In an early 90-day study of male C3H mice (Carpenter *et al.*, 1956) that were exposed to 0, 100, 200 or 400 ppm [0, 483, 966 or 1932 mg/m^3] 2-butoxyethanol (7 h per day on 5 days per week) for up to 90 days, transient haemoglobinuria and reversible increased liver weights were observed at the two higher concentrations, although no lesions were observed on microscopic examination of the liver, kidney and lung. The osmotic fragility of the red blood cells from all exposed animals was increased immediately after each exposure, but no increase in severity was apparent throughout the duration of the study. However, the erythrocytes returned to normal during the 17-h rest between exposures. Thus, the LOEL was found to be 100 ppm [483 mg/m^3].

No overt signs of toxicity and no effects on the weight or microscopic appearance of unspecified organs or on haematology (including osmotic fragility) were observed in New Zealand white rabbits that received daily dermal applications (covered) of up to 150 mg/kg bw per day 2-butoxyethanol for 6 h per day on 5 days per week for 13 weeks (Tyler, 1984; ECETOC, 1994, 1995).

(iii) *Chronic inhalation exposure*

A 2-year inhalation bioassay was conducted in groups of 50 male and 50 female Fischer 344/N rats that were exposed to 0, 31.2, 62.5 or 125 ppm [0, 151, 302 or 604 mg/m^3] 2-butoxyethanol (6 h per day on 5 days per week) for 104 weeks. An additional nine or 27 male and female rats per group were evaluated at 3, 6 and 12 months. Similar to the critical end-points observed in shorter-term studies, chronic exposure to 31.2 ppm [151 mg/m^3] 2-butoxyethanol (the lowest concentration tested) or more resulted in haemolytic anaemia (characterized as macrocytic, normochromic anaemia based on decreases in haematocrit, haemoglobin concentrations and erythrocyte counts, increases in mean cell volume and mean cell haemoglobin and the lack of an effect on mean cell haemoglobin concentration) in both male and female rats, which persisted throughout the 12 months that haematological parameters were monitored. The severity of these effects increased with level of exposure and did not improve over time. The anaemia was

considered to be responsive, based on the observation of increased reticulocyte and nucleated erythrocyte counts and a decrease in the myeloid:erythroid ratios. Consistent with results observed in earlier studies, toxicokinetic data indicated slower clearance of the active metabolite, 2-butoxyacetic acid, and greater activity of the relevant isoenzyme in females. In general, the severity of haematological effects was greater in females than in males, and alterations in multiple parameters were observed at the lowest concentration tested (i.e. 31.2 ppm [151 mg/m^3], which was considered to be the LOEL), while only mean cell volume was affected in males at this concentration (National Toxicology Program, 2000).

Neoplastic lesions were observed in the adrenal gland of female rats, as described in Section 3. A non-statistically significant increase in the incidence of hyperplasia of the adrenal medulla was also observed in females exposed to 125 ppm [302 mg/m^3]. No such increases were observed in males. Other exposure-related histopathological changes observed in rats exposed to 62.5 ppm and above included increased incidences of minimal hyaline degeneration of the olfactory epithelium in males and females (incidences: 13/48, 21/49, 23/49 and 40/50 males and 13/50, 18/48, 28/50 and 40/49 females exposed to 0, 31.2, 62.5 and 125 ppm, respectively) (which was considered by the authors to be an adaptive/protective effect rather than an adverse effect), increased incidences of pigmentation of Kupffer cells in the liver of males and females (incidences: 23/50, 30/50, 34/50 and 42/50 males and 15/50, 19/50, 36/50 and 47/50 females exposed to 0, 31.2, 62.5 and 125 ppm, respectively) and an increase in splenic fibrosis in males (incidences: 11/50, 14/50, 19/50 and 20/50 exposed to 0, 31.2, 62.5 and 125 ppm, respectively) (National Toxicology Program, 2000).

In a concurrent bioassay (National Toxicology Program, 2000), groups of 50 male and 50 female B6C3F$_1$ mice were exposed by inhalation to 0, 62.5, 125 or 250 ppm [0, 302, 604 or 1208 mg/m^3] 2-butoxyethanol for 6 h per day on 5 days per week for 104 weeks. An additional 30 male and 30 female mice were evaluated at 3, 6 and 12 months. Consistent with the results reported for shorter-term studies, B6C3F$_1$ mice were less sensitive to the haematological effects of 2-butoxyethanol than rats. Anaemia, which was characterized by decreases in haematocrit, haemoglobin concentrations and erythrocyte count, occurred at 3, 6 and 12 months in male and female mice exposed to the two higher concentrations (125 and 250 ppm [604 and 1208 mg/m^3]) and there was some evidence of anaemia in females exposed to 62.5 ppm [302 mg/m^3], but only at one time-point (6 months). In general, based on the lack of consistent changes in mean cell volume (except in females exposed to 250 ppm for 12 months) and mean cell haemoglobin concentrations, the effects observed in mice were consistent with normocytic, normochromic anaemia. Although it was considered to be responsive, based on the increased reticulocyte counts, this response improved over time. In addition, contrary to the observations in rats, there were no decreases in myeloid:erythroid ratios; in fact, increases were observed in some exposed groups. Thrombocytosis was present in both male and female mice at all concentrations, based on the increase in platelet counts; time of appearance was inversely related to concentration. As in rats, female mice were more sensitive than males, and significant altera-

tions in haematological parameters generally occurred earlier and at lower exposure levels in female mice.

The observed neoplastic lesions, including those of the forestomach, liver and circulatory system, are described in Section 3. The incidence of hyperplasia of the epithelium of the forestomach was significantly increased in a concentration-related manner in all exposed groups (1/50, 7/50, 16/50 and 21/50 males and 6/50, 27/50, 42/50 and 44/50 females exposed to 0, 62.5, 125 and 250 ppm, respectively), which was accompanied by a concentration-related trend in the incidence of ulcers of the forestomach in female mice (1/50, 7/50, 13/50 and 22/50). The authors hypothesized that the observed forestomach tumours represented a continuation of the injury/degeneration process, although the potential relationship between these lesions was not investigated. [The authors noted that the mechanism by which the forestomach is exposed is not clear; the role of preening or mucocilliary clearance of the respiratory tract in the exposure is unknown, although similar lesions and accumulation of 2-butoxyethanol in the forestomach of mice were also observed following oral or intraperitoneal administration (Poet *et al*., 2003).] Minimally severe haemosiderin pigmentation of Kupffer cells was also noted in the liver of exposed mice, which did not appear to be directly correlated to the incidence of neoplastic lesions in this organ, since such pigmentation was not present in all males in which liver tumours were observed but was noted in female mice that did not have an increased incidence of neoplastic lesions at this site. Therefore, although the pathogenesis of neoplastic lesions of the liver could not be determined, the authors suggested that it was unlikely to be related to the accumulation of haemosiderin pigment and was possibly related to oxidative stress. The LOEL for non-neoplastic effects (haematotoxicity and forestomach lesions) was 62.5 ppm [302 mg/m^3] in both sexes of mice.

(iv) *Other data*

Based on the limited data available, 2-butoxyethanol appears to have some immunomodulating potential, to which mice are more sensitive than rats. Significant effects on indicators of immune function were observed in BALB/c mice administered repeated oral doses of 50 mg/kg bw per day or more for 10 days (Morris *et al*., 1996), while only slight or no changes in immune function parameters were noted in Fischer 344 and Sprague-Dawley rats administered higher doses (i.e. up to 400 mg/kg bw per day by gavage for 2 days and up to 6000 pm in the drinking-water (equivalent to up to 444 mg/kg bw per day) for 21 days, respectively) (Exon *et al*., 1991; Smialowicz *et al*., 1992). Repeated dermal application of 500 mg/kg bw per day for 4 days or longer resulted in a reduced immune response of T cells, but not of B cells, in BALB/c mice (Singh *et al*., 2001); no similar studies in rats were identified. Topical administration of 4 mg 2-butoxyethanol resulted in a time-dependent decrease in contact hypersensitivity response to oxazolone in female BALB/c mice, whereas higher dermal doses or oral administration of up to 400 mg/kg bw per day for 10 days did not modulate this response (Singh *et al*., 2002). Reduced weights or histopathological changes were observed in the thymus or spleen of both mice and rats exposed subchronically or chronically to 2-butoxyethanol; however, it was considered that

these effects were probably secondary to haemolysis and decreased body weight (National Toxicology Program, 1993, 2000).

No investigations of the neurological effects of 2-butoxyethanol have been identified, although various signs of effects on the central nervous system, including loss of coordination, sluggishness, narcosis, muscular flaccidity and ataxia, have been reported after exposure to high doses or concentrations in numerous short-term studies (Carpenter *et al*., 1956; Dodd *et al*., 1983; Hardin *et al*., 1984; Krasavage, 1986).

(*c*) *In-vitro investigations of haemolytic effects*

Differences in species sensitivity to haemolysis induced by 2-butoxyethanol and its metabolites have been investigated in several studies *in vitro*. Consistent with the results of in-vivo studies, 2-butoxyacetic acid was more potent than either the parent compound or the acetaldehyde metabolite (Bartnik *et al*., 1987; Ghanayem, 1989; Sivarao & Mehendale, 1995). Although slight species differences were observed in erythrocytes exposed to 2-butoxyethanol (humans were less sensitive than rats, mice, dogs and guinea-pigs) (Carpenter *et al*., 1956; Bartnik *et al*., 1987), variability between species was much more pronounced when cells were exposed to 2-butoxyacetic acid (erythrocytes from humans were less sensitive than those of rats). In a comparison across multiple mammalian species (three to five animals per species), Ghanayem and Sullivan (1993) observed that red blood cells from rats, mice, hamsters, rabbits and baboons were more sensitive to the effects of 2-butoxyacetic acid than those of pigs, dogs, cats, guinea-pigs and humans.

Bartnik *et al.* (1987) reported that 1.25 mM [165 μg/mL] 2-butoxyacetic acid (the lowest concentration administered) resulted in 25% haemolysis in male Wistar rat erythrocytes after 180 min, while, in contrast, 15 mM [1980 μg/mL] 2-butoxyacetic acid did not produce measurable haemolysis in erythrocytes from healthy humans over the same time. [This study was conducted on washed erythrocytes rather than whole blood, which indicates that the species difference in sensitivity *in vitro* was probably due to an inherent difference in the erythrocytes, rather than in the extent of plasma protein binding of 2-butoxyacetic acid.] Ghanayem (1989) exposed pooled whole blood from male Fischer 344 rats and healthy human volunteers (three men and women) to 2-butoxyacetic acid for 0.25–4 h and measured haematocrit and free plasma haemoglobin levels as indicators of swelling of the erythrocytes and haemolysis, respectively. Based on observations after 4 h of incubation, 8.0 mM [1056 μg/mL] 2-butoxyacetic acid had less effect in humans than 0.5 mM [66 μg/mL] 2-butoxyacetic acid had in rats. [However, it is not clear from the data presented whether the slight changes in human erythrocytes were produced in relation to the initial control value or were compared with data for a 4-h incubation in the absence of 2-butoxyacetic acid.] Similarly, Udden and Patton (1994) observed no effects in human erythrocytes exposed to 2 mM [264 μg/mL] 2-butoxyacetic acid (the maximum concentration tested) for 4 h, although this concentration induced rapid haemolysis in rat erythrocytes. Exposure of rat erythrocytes to 0.2 mM [26.4 μg/mL] 2-butoxyacetic acid did not result in haemolysis, although reduced cell deformability and increased mean cell volume were noted. In a subsequent investigation, similar sub-haemolytic changes were

observed, including loss of deformability and an increase in mean cell volume and cell sodium levels, in human erythrocytes (from up to 14 individuals) that were incubated with 7.5 or 10.0 mM [990 or 1320 µg/mL] 2-butoxyacetic acid and in rat erythrocytes exposed to lower concentrations of 2-butoxyacetic acid (i.e. 0.05, 0.075 or 1.0 mM [6.6, 9.9 or 132 µg/mL]). A slight, but statistically significant increase in haemolysis was observed in red blood cells obtained from 40 hospitalized adults that were exposed *in vitro* to 10 mM 2-butoxyacetic acid; significant increases in haemolysis were not noted in exposed cells from 11 healthy adults or 46 hospitalized children (Udden, 2002).

Following incubation for 4 h with 2 mM [264 µg/mL] 2-butoxyacetic acid, Udden (1994) reported a lack of haemolysis in human red blood cells from groups of different human subjects, including nine healthy young adults (31–56 years old), nine older subjects (64–79 years old), seven patients who had sickle-cell disease and three patients who had spherocytosis. However, these groups differed with regard to the extent of spontaneous haemolysis that occurred after incubation of their red blood cells for 4 h in the absence of 2-butoxyacetic acid.

Unlike the sex-related differences in sensitivity to 2-butoxyethanol-induced haematological effects that have been observed in rats exposed *in vivo*, in-vitro exposure of rat erythrocytes to the metabolite 2-butoxyacetic acid revealed no differences in sensitivity between males and females, as measured by packed cell volume (Ghanayem *et al*., 2000), which provides further support to the supposition that differences *in vivo* are related to differences in metabolism and toxicokinetics of 2-butoxyethanol rather than to differences in toxicodynamics.

(*d*) *Mode of action that induces haematological effects*

Extensive data from comparative toxicity studies with the parent compound and its metabolites, as well as from studies in which oxidation of 2-butoxyethanol to 2-butoxyacetic acid is inhibited, indicate that 2-butoxyacetic acid is the metabolite that is principally responsible for the haematological effects observed in experimental animals exposed to 2-butoxyethanol (Ghanayam *et al*., 1987b; Morel *et al*., 1996). The changes in haematological parameters induced by 2-butoxyacetic acid are characteristic of haemolytic anaemia, although the mode(s) of action by which 2-butoxyacetic acid induces these effects has (have) not been established conclusively. Before the occurrence of haemolysis in red blood cells from rats, erythrocyte swelling, morphological changes (including a change from typical discocytic to spherocytic appearance and stomatocytosis) and decreased deformability have been observed (Udden, 1996). Udden (2000) observed similar morphological changes in rat erythrocytes following in-vivo exposure to 125 mg/kg bw 2-butoxyethanol and in-vitro exposure to 1.0 mM [132 µg/mL] 2-butoxyacetic acid which are suggestive of a progression from stomatocytosis, or cupping, to spherocytosis and, finally, to lysis. These morphological changes were not observed in human erythrocytes incubated with up to 2.0 mM [264 µg/mL] 2-butoxyacetic acid. The severity of morphological changes in erythrocytes, including stomatocytosis, macrocytosis, moderate rouleaux formation, spherocytosis, schistocytosis and ghost cell forma-

tion, increased progressively in male and female Fischer 344 rats administered 250 mg/kg bw 2-butoxyethanol per day by gavage for 1, 2 or 3 days (Ghanayem *et al.*, 2001). Based on available information from his own research and the investigations of others, Ghanayem (1996) hypothesized that the swelling of erythrocytes was due to increased cell membrane permeability to cations and water and noted that such changes in cellular membranes have been observed in the blood of humans who have hereditary spherocytosis, as have stomatocytes, which are associated with altered intracellular levels of sodium and potassium that lead to increased cell water, increased mean cell volume, decreased deformability and increased osmotic fragility. 2-Butoxyacetic acid-induced swelling of rat erythrocytes exposed *in vitro* continued after removal of 2-butoxyacetic acid and was not reversible, although the rate of swelling decreased (Ghanayem *et al*., 1989). These authors also investigated the partitioning of 2-butoxyacetic acid between erythrocytes and plasma and determined that the concentration in cells increased over time while that in the plasma remained relatively constant (Ghanayem *et al.*, 1989). In a previous study, Ghanayem and Matthews (1990) showed that administration of calcium channel blockers to rats before exposure to 2-butoxyethanol decreased the erythrocyte swelling and improved the associated haemolytic anaemia; it was speculated that homeostasis of intracellular calcium or other cations (whose passage through cellular membranes may be affected by such blockers) may be involved in 2-butoxyethanol-induced toxicity (Ghanayem, 1996). Conjugation of 2-butoxyethanol with long-chain fatty acids in the liver has been observed in rats (Kaphalia *et al*., 1996), although it is not known whether such conjugation would occur when lipids were present in erythrocyte membranes. In addition, Dartsch *et al*. (1999) reported that the intermediate metabolite, butoxyacetaldehyde, caused depolymerization of intracellular stress fibres that contain actin in mammalian renal epithelial cells, which resulted in morphological alterations in cell shape and volume that were consistent with the hypothesis that cell swelling points to the induction of a necrotic process.

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data on the potential reproductive or developmental effects of 2-butoxyethanol in humans were available to the Working Group.

4.3.2 *Experimental systems*

(*a*) *Reproductive toxicity*

Very limited information on the potential reproductive toxicity of 2-butoxyethanol is available. In the only investigation of the potential effect of 2-butoxyethanol on reproductive ability identified (i.e. the multigeneration study by Heindel *et al.* (1990)), administration of 700–2100 mg/kg bw per day 2-butoxyethanol in the drinking-water to breeding

pairs of CD-1 mice throughout mating, pregnancy and lactation did not induce any discernible effects on male reproductive organs or sperm or on the estrous cycle of females. However, fertility was impaired, based on statistically significant reductions in the numbers of litters per pair and live pups per litter after exposure to 1300 and 2100 mg/kg bw per day; these doses were also associated with decreased body-weight gain and drinking-water consumption in the parent mice. Based on the results of a cross-over mating trial, in which exposed males were mated with control females and exposed females were mated with control males, these effects were found to be primarily due to an effect on the treated females. Because of the lack of sufficient pups in the groups treated with 1300 and 2100 mg/kg bw per day, fertility was only examined in the group that was administered 700 mg/kg bw per day. No effects on reproductive ability, as measured by mating and fertility indices, litter size, proportion of live pups or live pup weights, were noted in mice exposed to 2-butoxyethanol *in utero*, from weaning to sexual maturity or until delivery of a litter.

Acute exposure to 800 ppm [3864 mg/m^3] of a saturated vapour of 2-butoxyethanol for 3 h did not result in altered testicular weight in Alpk/Ap rats (Doe, 1984). Similarly, no histopathological effects on the testes, epididymidis or seminal vesicles were observed in Alpk/Ap rats administered a single oral dose of up to 868 mg/kg bw butoxyacetic acid by gavage (Foster *et al*., 1987).

No effects on testes weight or histopathology were reported in short-term studies in which Fischer 344 (or Fischer 344/N), Sprague-Dawley and Crl:COBS CD (SD)BR rats were administered doses of up to 1000, 506 and 885 mg/kg bw 2-butoxyethanol per day, respectively, and JCL-ICR mice were administered up to 2000 mg/kg bw per day (Nagano *et al*., 1979, 1984; Grant *et al*., 1985; Krasavage, 1986; Exon *et al*., 1991; National Toxicology Program, 1993).

Sperm morphology and vaginal cytology were assessed in Fischer 344/N rats and B6C3F$_1$ mice that received 2-butoxyethanol in the drinking-water for 13 weeks. In mice, no evidence was found of an adverse effect on the estrous cycle of females administered up to 1306 mg/kg bw per day, while a slight reduction in sperm motility was observed in males that received 694 mg/kg bw per day and absolute left testis weights were slightly reduced at all dose levels (i.e. 553 mg/kg bw per day and above). In the corresponding study in rats, uterine atrophy or decreased sperm concentration were evident, with LOELs of 304 and 281 mg/kg bw per day in females and males, respectively (National Toxicology Program, 1993).

Testicular degeneration and necrosis of the epididymis was noted in a subchronic study in B6C3F$_1$ mice that were exposed by inhalation to 500 ppm [2415 mg/m^3] 2-butoxyethanol, a concentration that was associated with decreased survival and lesions in several organs (National Toxicology Program, 2000). No such effects were noted in the concurrent study in Fischer 344/N rats exposed to up to 500 ppm 2-butoxyethanol.

No effects on male or female reproductive organs were reported in a 2-year bioassay in which Fischer 344/N rats and B6C3F$_1$ mice were exposed to up to 125 and 250 ppm [604 and 1208 mg/m^3] 2-butoxyethanol, respectively (National Toxicology Program, 2000).

(*b*) *Developmental toxicity*

In studies in rats and mice, oral administration of 2-butoxyethanol during pregnancy induced fetotoxic and/or embryotoxic effects, but generally only at or above maternally toxic doses. When Fischer 344 rats were administered 0, 30, 100 or 200 mg/kg bw 2-butoxyethanol per day by gavage on days 9–11 of pregnancy (National Toxicology Program, 1989), increased fetal deaths occurred after exposure to 200 mg/kg bw per day. Maternal toxicity (including haemolysis) was evident at doses of 100 mg/kg bw per day or more. The frequency of malformations was not increased at any dose level. Haematological effects (reduced platelet count) were also noted in fetuses of dams that were exposed to 300 mg/kg bw per day on days 11–13 of gestation, although inconsistent changes in mean corpuscular volume were observed at lower doses. The LOELs for developmental and maternal toxicity were considered to be 200 and 100 mg/kg bw per day, respectively; no effects were noted after exposure to 30 mg/kg bw per day.

Pregnant CD-1 mice were administered 0, 350, 650, 1000, 1500 or 2000 mg/kg bw 2-butoxyethanol per day by gavage on days 8–14 of gestation (Wier *et al.*, 1987). An increase in the number of resorbed embryos was observed after oral administration of 1000 mg/kg bw per day or more. Clinical signs of toxicity were observed in dams (staining of cage papers, lethargy, abnormal breathing, failure to right or mortality) treated with 650 mg/kg bw per day and above. The LOEL for maternal toxicity and the no-observed-adverse-effect level (NOAEL) for developmental toxicity were considered to be 650 mg/kg bw per day. In pups of dams administered 650 or 1000 mg/kg bw per day, no effects on survival or growth as of postnatal day 22 were observed. Administration by gavage of 1180 mg/kg bw per day (only dose tested) to CD-1 mice during days 7–14 of pregnancy resulted in maternal deaths (20%) and reductions in the number of viable litters, but did not affect pup weight or postnatal survival (Schuler *et al.*,1984; Hardin *et al.*, 1987).

Heindel *et al.* (1990) observed a slight fetotoxic effect at a dose that was not overtly toxic to the parent animals; in a multigeneration study, a small, but statistically significant reduction in live pup weight (by 4.3%) was observed in the F_1 generation after administration of 0.5% 2-butoxyethanol in the drinking-water (equivalent to 700 mg/kg bw per day) to male and female CD-1 mice throughout mating, pregnancy and lactation. Greater reductions in live pup weight were noted at higher doses (1 and 2% in the drinking-water; equivalent to 1300 or 2100 mg/kg bw per day) at which overt maternal toxicity (decreased body weight and fluid consumption, increased kidney weight and/or increased mortality) was observed. This effect was attributed to maternal exposure, since it was only observed in pairings in which exposed females were mated with control males. However, although a 4.4% reduction in live pup weight was noted in the F_2 generation, this decrease was not statistically significant (which indicated that the effect did not worsen in successive generations), and 700 mg/kg bw per day was considered by the investigators to be close to the NOAEL for developmental toxicity.

Developmental toxicity in the form of increased numbers of non-viable implants and resorptions and decreased percentage of live fetuses per litter was observed in Fischer 344 rats exposed to 200 ppm [966 mg/m^3] 2-butoxyethanol on days 6–15 of pregnancy. Reduced skeletal ossification was evident at doses of 100 ppm [483 mg/m^3] or more (concentrations that were also maternally toxic based on weight loss and haematological effects). Therefore, the LOEL for maternal and developmental toxicity was considered to be 100 ppm, since no effects were observed at doses of 25 or 50 ppm [121 or 242 mg/m^3] (Tyl *et al*., 1984). However, no significant developmental effects occurred in Sprague-Dawley rats exposed to 150 or 200 ppm [726 or 966 mg/m^3] 2-butoxyethanol on days 7–15 of pregnancy, although haematuria occurred in dams on the first day of exposure at 200 ppm (Nelson *et al*., 1984). In rabbits exposed to 2-butoxyethanol on days 6–18 of pregnancy, the LOEL for both maternal and developmental toxicity was 200 ppm [966 mg/m^3], based on increased mortality, abortions and weight loss and a slight reduction in the number of viable implants per litter; no significant toxicity was noted in dams or fetuses at doses of 100 ppm [483 mg/m^3] or less (Tyl *et al*., 1984).

In a study of Sprague-Dawley rats that received repeated dermal applications (not specified if the site of application was covered) of neat 2-butoxyethanol on days 7–16 of pregnancy, no overt signs of maternal toxicity and no evidence of developmental toxicity were observed at doses of 0.48 mL per day (~3.6 mmol per day; ~1920 mg/kg bw per day). However, the higher dose of 1.4 mL per day (~10.8 mmole per day; ~5600 mg/kg bw per day) was lethal to the dams (Hardin *et al.*, 1984).

4.4 Genetic and related effects

The genetic toxicology of 2-butoxyethanol and its major metabolites, 2-butoxyacetaldehyde and 2-butoxyacetic acid, has been reviewed (Elliott & Ashby, 1997).

4.4.1 *Humans*

The potential genotoxicity of 2-butoxyethanol and other glycol ethers was studied in a group of 19 workers (15 men and four women) who were occupationally exposed to glycol ethers in a varnish production plant. External and internal exposures were assessed by personal air monitoring on Monday and Tuesday after an exposure-free weekend. In the varnish production area, the concentrations of 2-butoxyethanol, 2-ethoxyethanol and 2-ethoxyethyl acetate in air averaged 0.5, 2.9 and 0.5 ppm, respectively, on Monday and 0.6, 2.1 and 0.1 ppm, respectively, on Tuesday. At the same workplaces, the mean concentrations of 2-butoxyacetic acid and 2-ethoxyacetic acid in the urine of workers were 0.2 and 53.2 mg/L on Monday before the workshift and 16.4 and 53.8 mg/L on Tuesday after the workshift, respectively. On Tuesday after the shift, venous blood samples were collected from the workers, and lymphocyte cultures were prepared and analysed for the frequency of sister chromatid exchange and micronuclei. Fifteen persons who were not occupationally exposed to glycol ethers and who largely matched the exposed group in

terms of age and smoking habits served as controls. A comparison of all exposed and unexposed workers as well as of the small subgroups of exposed and unexposed smokers and nonsmokers did not show any increase in the mean frequencies of sister chromatid exchange or micronuclei in the exposed workers (Söhnlein *et al.*, 1993).

No data on the genetic and related effects of 2-butoxyacetaldehyde or 2-butoxyacetic acid in humans were available to the Working Group.

4.4.2 *Experimental systems* (see Table 17 for details and references)

2-Butoxyethanol

(*a*) *In-vitro studies*

2-Butoxyethanol has been examined in several bacterial mutagenicity assays both in the absence and in the presence of an exogenous metabolic activation system. In one of these studies (Zeiger *et al.* 1992), postmitochondrial supernatant from both rats and hamsters was used. No induction of gene mutations by 2-butoxyethanol was observed in *Escherichia coli* strain WP2*uvrA* or in *Salmonella typhimurium* strains TA100, TA102, TA1535, TA1537, TA98 or TA97. In a single study, mutagenic activity of 2-butoxyethanol in strain TA97a, which is closely related to strain TA97, was reported. This finding could not be confirmed, however, in a thorough independent study that was specifically designed to investigate this observation. Thus, there is no convincing evidence that 2-butoxyethanol can cause gene mutation in bacteria.

2-Butoxyethanol was also ineffective in inducing mutations in a bacteriophage assay.

In rat and mouse hepatocytes, 2-butoxyethanol did not cause a significant increase in the level of 8-hydroxydeoxyguanosine in DNA, a biomarker of oxidative stress.

In an assay for the induction of mutations in mammalian cells *in vitro*, 2-butoxyethanol did not increase the frequency of *Gpt* mutations in Chinese hamster ovary (CHO-AS52) cells, a subline that has been genetically engineered to allow the detection of both base substitutions and deletions that may result from clastogenic events. In another study, 2-butoxyethanol was reported to induce an increase in mutant frequency in Chinese hamster V79 cells over the concentration range of 20–75 mmol/L [2360–8850 µg/mL]. However, no data and no information on cytotoxicity were presented, and the description was inadequate for evaluation (Elias *et al.*, 1996).

Studies on the capacity of 2-butoxyethanol to induce sister chromatid exchange have yielded inconsistent results. Whereas negative results were reported in Chinese hamster ovary cells, positive findings were obtained in Chinese hamster lung V79 cells and human lymphocytes. A cell cycle delay was observed in 2-butoxyethanol-treated V79 cells (Elias *et al.*, 1996). [As no details on cell cycle kinetics of treated and untreated cells were given for the V79 cells and no data on toxicity or cell cycle were presented for human lymphocytes, the Working Group noted that whether the positive effects reported for these sister chromatid exchange assays are artefacts or not cannot be fully evaluated.]

Table 17. Genetic and related effects of 2-butoxyethanol and its metabolites

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
2-Butoxyethanol				
Escherichia coli WP2*uvrA*, reverse mutation	–	–	10 000 µg/plate	Gollapudi *et al*. (1996)
Salmonella typhimurium TA100, TA1535, TA1537, TA98,TA97, reverse mutation	–	–	10 000 µg/plate	Zeiger *et al*. (1992)
Salmonella typhimurium TA100, TA102, TA98, reverse mutation	–	–	13 600 µg/plate	Hoflack *et al*. (1995)
Salmonella typhimurium TA100, TA97a, reverse mutation	–	–	10 000 µg/plate	Gollapudi *et al*. (1996)
Salmonella typhimurium TA97a, reverse mutation	+	NT	2200	Hoflack *et al*. (1995)
Salmonella typhimurium TA97a, reverse mutation	NT	+	9000 µg/plate	Hoflack *et al*. (1995)
Bacteriophage *T4D*, mutation	–	NT	111.1	Kvelland (1988)
Oxidative DNA damage, 8-OHdG, B6C3F$_1$ mouse and Fischer 344 rat hepatocytes *in vitro*	–	NT	2954	Park *et al.* (2002a)
Gene mutation, Chinese hamster ovary (CHO-AS52) cells, *Gpt* locus *in vitro*	–	NT	898	Chiewchanwit & Au (1995)
Gene mutation, Chinese hamster V79 cells, *Hprt* locus *in vitro*	(+)	NT	2363[c]	Elias *et al.* (1996)
Sister chromatid exchange, Chinese hamster ovary (CHO) cells *in vitro*	–	NT	3500	National Toxicology Program (1993)
Sister chromatid exchange, Chinese hamster ovary (CHO) cells *in vitro*	NT	–	5 000	National Toxicology Program (1993)
Sister chromatid exchange, Chinese hamster V79 cells *in vitro*	(+)	NT	2363[c]	Elias *et al.* (1996)
Micronucleus formation, Chinese hamster V79 cells *in vitro*	(+)	NT	1005	Elias *et al.* (1996)
Chromosomal aberrations, Chinese hamster ovary (CHO) cells *in vitro*	–	–	5000	National Toxicology Program (1993)
Chromosomal aberrations, Chinese hamster V79 cells *in vitro*	–	NT	NG	Elias *et al.* (1996)
Aneuploidy, Chinese hamster V79 cells *in vitro*	+	NT	993	Elias *et al.* (1996)
Cell transformation, Syrian hamster embryo cells	–	NT	NG	Elias *et al.* (1996)
Cell transformation, Syrian hamster embryo cells	(+)	NT	1000	Kerckaert *et al.* (1996)
Cell transformation, Syrian hamster embryo cells	–	NT	2363	Park *et al.* (2002b)

Table 17 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Sister chromatid exchange, human peripheral lymphocytes *in vitro*	+		500 ppm [500]	Villalobos-Pietrini *et al.* (1989)
Chromosomal aberrations, human peripheral lymphocytes *in vitro*	–	–	3000 ppm [3000]	Villalobos-Pietrini *et al.* (1989)
Chromosomal aberrations, human peripheral lymphocytes *in vitro*	–	NT	NG	Elias *et al.* (1996)
Oxidative DNA damage, 8-OHdG, mouse liver *in vivo*	+		900 po, 7 d	Park *et al.* (2002a)
Oxidative DNA damage, 8-OHdG, mouse liver *in vivo*	+		450 po × 5/wk, 7–90 d	Siesky *et al.* (2002)
Oxidative DNA damage, 8-OHdG, rat liver *in vivo*	–		450 po × 5/wk, 7–90 d	Siesky *et al.* (2002)
DNA adduct formation (^{32}P-postlabelling), Sprague-Dawley rats, brain, kidney, liver, spleen, testes *in vivo*	–		120 po × 1	Keith *et al.* (1996)
Micronucleus formation, male and female CD-1 mice, polychromatic erythrocytes in bone marrow *in vivo*	–		800 ip × 1	Elias *et al.* (1996)
Micronucleus formation, male B6C3F$_1$ mice, polychromatic erythrocytes in bone marrow *in vivo*	–		550 ip × 3	National Toxicology Program (2000)
Micronucleus formation, male Fischer 344/N rats, polychromatic erythrocytes in bone marrow *in vivo*	–		450 ip × 3	National Toxicology Program (2000)
Inhibition of intercellular communication, Chinese hamster V79 cells *in vitro*	+	NT	1005	Elias *et al.* (1996)
2-Butoxyacetaldehyde				
Salmonella typhimurium TA100, TA102, TA98, TA97a, reverse mutation	–	–	5000 µg/plate	Hoflack *et al*. (1995)
Gene mutation, Chinese hamster ovary (CHO-AS52) cells, *Gpt* locus *in vitro*	–	NT	0.2 % (v/v) 2000	Chiewchanwit & Au (1995)
Gene mutation, Chinese hamster V79 cells, *Hprt* locus *in vitro*	+	NT	232	Elias *et al.* (1996)
Sister chromatid exchange, Chinese hamster V79 cells *in vitro*	+	NT	23.2	Elias *et al.* (1996)

Table 17 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Micronucleus formation, Chinese hamster V79 cells *in vitro*	+	NT	4.7	Elias *et al.* (1996)
Chromosomal aberrations, Chinese hamster V79 cells *in vitro*	+	NT	9.5[c]	Elias *et al.* (1996)
Aneuploidy, Chinese hamster V79 cells *in vitro*	+	NT	10.6	Elias *et al.* (1996)
Cell transformation, Syrian hamster embryo cells *in vitro*	–	NT	NG	Elias *et al.* (1996)
Inhibition of intercellular communication, Chinese hamster V79 cells *in vitro*	–	NT	10	Elias *et al.* (1996)
Chromosomal aberrations, human peripheral lymphocytes *in vitro*	+	NT	9.5[c]	Elias *et al.* (1996)
2-Butoxyacetic acid				
Salmonella typhimurium TA100, TA102, TA98, TA97a, reverse mutation	–	–	1 000 µg/plate	Hoflack *et al*. (1995)
Sister chromatid exchange, Chinese hamster V79 cells *in vitro*	–	NT	105[c]	Elias *et al.* (1996)
Micronucleus formation, Chinese hamster V79 cells *in vitro*	(+)	NT	660	Elias *et al.* (1996)
Chromosomal aberrations, Chinese hamster V79 cells *in vitro*	–	NT	NG	Elias *et al.* (1996)
Aneuploidy, Chinese hamster V79 cells *in vitro*	?	NT	50	Elias *et al.* (1996)
Cell transformation, Syrian hamster embryo cells	–	NT	NG	Elias *et al.* (1996)
Chromosomal aberrations, human peripheral lymphocytes *in vitro*	–	NT	NG	Elias *et al.* (1996)
Micronucleus formation, male and female CD-1 mice, polychromatic erythrocytes in bone marrow *in vivo*	–		200 ip × 1	Elias *et al.* (1996)
Inhibition of intercellular communication, Chinese hamster V79 cells *in vitro*	–	NT	12 408	Elias *et al.* (1996)

8-OHdG, 8-hydroxydeoxyguanosine

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

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

[c] Estimated from the graph in the article

Consistently negative results were obtained in three independent studies that assessed the ability of 2-butoxyethanol to induce structural chromosomal aberrations in Chinese hamster ovary (CHO) cells and V79 lung cells and human lymphocytes.

In an in-vitro study that examined several genetic or related end-points in Chinese hamster V79 cells, a weak induction of micronuclei, an increased percentage of hyperdiploid cells (aneuploidy) and inhibition of gap-junctional metabolic cooperation between *Hprt*$^{+}$ and *Hprt*$^{-}$ cells were observed following treatment of the cells with 2-butoxyethanol. These assays are known to be especially sensitive to interference by the cytotoxic effects of test agents. [The Working Group felt that, in view of the lack of information provided on the toxicity of the treatments, the description of the results was too limited for an evaluation.]

Tests for the ability of 2-butoxyethanol to induce morphological transformation of Syrian hamster embryo cells yielded inconclusive results. Negative results were obtained in two studies in which the cells were incubated with the test compound for 7 days. Positive results were reported in an assay that used an exposure period of 24 h. [The Working Group noted that an inconsistent concentration–effect relationship was evident in this experiment.]

(*b*) *In-vivo studies*

In mice exposed to 2-butoxyethanol by repeated oral administration of 450 and 900 mg/kg bw for 7 days, significantly elevated levels of 8-hydroxydeoxyguanosine were observed in hepatic DNA. This effect was attributed by the authors to oxidative stress induced via the induction of haemolysis and a resulting increase in iron deposition in the liver (Park *et al.*, 2002a). No increase in 8-hydroxydeoxyguanosine was found in liver DNA of similarly exposed rats. This difference is consistent with the species differences in the development of hepatic tumours (see Section 3). No evidence for the genotoxic activity of 2-butoxyethanol was obtained in other in-vivo studies. Oral administration of 120 mg/kg bw to rats did not cause the formation of hydrophilic or hydrophobic DNA adducts detectable by ^{32}P-postlabelling in the brain, liver, kidney spleen or testes. 2-Butoxyethanol did not increase the frequency of micronucleated polychromatic erythrocytes in the bone marrow of mice and rats following repeated intraperitoneal applications or of mice following a single intraperitoneal application. The ratio of polychromatic and normochromatic erythrocytes, a potential indicator of treatment-related cytotoxic effects on the bone marrow, was only determined in the study that used a single application and was found to be unaltered.

(i) *Alterations of proto-oncogenes in tumours*

In order to determine whether the induction of mutations in proto-oncogenes by 2-butoxyethanol may have been involved in the formation of forestomach neoplasms induced by this compound in male and female B6C3F$_1$ mice (see Section 3), the spectra of H-*ras* proto-oncogene mutations in forestomach tumours of animals exposed to 2-butoxyethanol and of control animals were compared. Fourteen tumours from exposed

mice and 11 from control mice were analysed. Codon 61 mutations in the H-*ras* gene were detected in 57% of the tumours of the exposed animals and in 45% of those of the control animals. The mutation profiles within the forestromach neoplasms of the 2-butoxyethanol-exposed animals did not differ significantly from those in the spontaneous neoplasms. The high frequency of activated H-*ras* gene that was detected in spontaneous neoplasms suggested that this gene is important for the formation of forestomach tumours in $B6C3F_1$ mice. The findings indicate that 2-butoxyethanol does not increase the mutation frequency of the H-*ras* proto-oncogene and suggest that the compound may enhance tumour formation by promoting clonal growth of spontaneously initiated forestomach cells that primarily contain an activated H-*ras* gene (National Toxicology Program, 2000).

In a related study, the effect of 2-butoxyethanol on the level of DNA methylation was investigated in mice and rats. The continuous treatment of transgenic FVB/N mice (Oncomice Neo 01^{TM}) which carry the viral Harvey *ras* (v-Ha-*ras*) oncogene with ~120 mg/kg bw 2-butoxyethanol per day via subcutaneously implanted minipumps for 2 weeks did not result in an alteration of the methylation status of the Ha-*ras* transgene in testes, spleen, brain, kidney or liver within 5 and 120 days after the start of administration. The authors concluded that it appears unlikely that 2-butoxyethanol causes demethylation of DNA, which would increase the expression of v-Ha-*ras*, or increased methylation of DNA, which would induce mutations through the formation of thymine from 5-methylcytosine. A ^{32}P-postlabelling study of the level of 5-methylcytosine from whole genome analyses of male and female v-Ha-*ras* transgenic mice treated with 2-butoxyethanol (minipump for up to 120 days) and of male Sprague-Dawley rats (120 mg/kg bw by gavage for 1 day) did not reveal any effect of the compounds on total DNA methylation in mice (brain, bone marrow, spleen and testes) or rats (brain, kidney, liver, spleen and testes) (Keith *et al.*, 1996).

(ii) *Interference with DNA repair*

High concentrations (≥ 8.5 mM) of 2-butoxyethanol have been shown to potentiate the induction of chromosomal aberrations in Chinese hamster V79 cells by methyl methanesulfonate (Elias *et al.*, 1996). As 2-butoxyethanol alone did not display any clastogenic effect (Elias *et al.*, 1996), it was hypothesized that the compound might inhibit the repair of DNA that was previously damaged by the alkylating agent. In accordance with this hypothesis, Hoflack *et al.* (1997) observed that subsequent exposure to 2-butoxyethanol (5 mM) of Syrian hamster embryo cells that had been treated with methyl methanesulfonate (0.2 mM), which rapidly increases the cellular concentration of poly(ADP-ribose), inhibited poly(ADP-ribose) synthesis. No effect of 2-butoxyethanol on poly(ADP-ribose) concentration was observed in cells not pretreated with methyl methanesulfonate. The decrease was not caused by a depletion of NAD^+, the substrate of the polymerization reaction, by 2-butoxyethanol, and the underlying mechanism remained unclear. As poly(ADP-ribosyl)ation is involved in base-excision repair of DNA damage, this observation supports the notion that high concentrations of 2-butoxyethanol may interfere with the repair of certain types of DNA lesions and the maintenance of chromosomal stability.

2-Butoxyethanol metabolites

(*a*) *2-Butoxyacetaldehyde*

2-Butoxyacetaldehyde did not induce gene mutations in several strains of *S. typhimurium* in the absence or presence of an exogenous metabolic activation system. It was not mutagenic in the Chinese hamster ovary cell subline CHO-AS52, in which the shorter analogue of 2-butoxyacetaldehyde, methoxyacetaldehyde, had been shown to induce mutations (Ma *et al.*, 1993). However, one study reported genotoxic effects at comparatively low concentrations of 2-butoxyacetaldehyde in various assays in Chinese hamster V79 cells without exogenous metabolic activation (an assay for gene mutations at the *Hprt* locus, an assay for the induction of sister chromatid exchange and an assay for the induction chromosomal aberrations, micronucleus formation and aneuploidy). Tests for the inhibition of metabolic cooperation of Chinese hamster V79 cells and for the transformation of Syrian hamster embryo cells yielded negative results. The results of this study may be taken as an indication of a weak genotoxic activity of 2-butoxyacetaldehyde that appears to be predominantly expressed at the chromosomal level. [The Working Group felt that the presentation of the results did not allow a critical examination of the data.]

(*b*) *2-Butoxyacetic acid*

2-Butoxyacetic acid is an aliphatic acid and would not be expected to exhibit any genotoxic activity. The compound did not induce mutations in several strains of *S. typhimurium* in the absence or presence of an exogenous metabolic activation system. In a series of in-vitro assays performed without an exogenous metabolic activation system, it did not increase the frequency of sister chromatid exchange or of structural chromosomal aberrations and did not inhibit gap-junctional metabolic cooperation in Chinese hamster V79 cells. In contrast, weakly positive results were found in a micronucleus assay with V79 cells and, in the same cell line, a weak effect of 2-butoxyacetic acid on the percentage of aneuploid cells was reported. 2-Butoxyacetic acid did not increase the frequency of chromosomal aberrations in human lymphocytes *in vitro*. [The Working Group felt that the limited data presented did not allow any conclusion on the extent to which cytotoxic effects of the treatment or pH changes induced by the acid in culture medium may have contributed to the results reported.]

In the only available in-vivo assay of 2-butoxyacetic acid, the compound did not affect the frequency of micronucleated polychromatic erythrocytes in the bone marrow of mice following a single intraperitoneal injection. In this study, 2-butoxyacetic acid was toxic to the bone marrow, as statistically significant decreases in the ratio of polychromatic and normochromatic erythrocytes were observed at the two highest doses (100 and 200 mg/kg bw).

4.5 Mechanistic considerations

The available data on 2-butoxyethanol largely support the concept that the compound, the structure of which does not carry any alerts for probable genotoxic activity (Ashby &

Tennant, 1991; Tennant & Ashby, 1991; Elliott & Ashby, 1997), exhibits no appreciable genotoxic effects. Although many studies were carried out in the absence of metabolic activation, consistent data show that 2-butoxyethanol does not induce gene mutations in bacteria or structural chromosomal aberrations in mammalian cells *in vitro*, and there is evidence that the compound does not cause oxidative stress that results in genotoxicity in hepatocytes *in vitro*. Weakly positive results have been reported for high concentrations of 2-butoxyethanol in some in-vitro studies of cultured mammalian cells, but the way in which the data were presented and the lack of essential information on experimental details cause uncertainties that prevent their full evaluation. It cannot be excluded, however, that some of the positive findings actually represent true genotoxic effects caused by the metabolite, 2-butoxyacetaldehyde, which may be formed in small amounts *in vitro* depending on the concentration of 2-butoxyethanol, the type of cells and the exogenous metabolic activation system used. Although speculative, this is consistent with a number of potentially positive results that were reported for 2-butoxyacetaldehyde in some assays that mainly used cytogenetic end-points. The genotoxic activity of this aldehyde appears to be lower, however, than that of other glycol ether-derived aldehydes with shorter alkyl groups, since methoxyacetaldehyde is mutagenic in Chinese hamster CHO-AS52 cells and clastogenic in standard Chinese hamster ovary cells, whereas 2-butoxyacetaldehyde is not (Ma *et al.*, 1993; Chiewchanwit & Au, 1995). The metabolic end-product of the oxidation of 2-butoxyethanol and 2-butoxyacetaldehyde, 2-butoxyacetic acid, appears to be non-genotoxic.

Several in-vivo assays with 2-butoxyethanol have shown the absence of detectable genotoxic activity in bone marrow. Studies on the effects of 2-butoxyethanol on mouse liver have indicated, however, that it causes oxidative stress associated with the formation of oxidative base damage in hepatic DNA, as demonstrated by increased levels of 8-hydroxydeoxyguanosine and other biomarkers of oxidative stress (Park *et al.*, 2002a). This hepatic oxidative stress has not been attributed to the action of 2-butoxyethanol *per se*, but to the metabolic formation of 2-butoxyacetic acid via the induction of haemolysis in the liver (see below).

The haematological effects of 2-butoxyethanol have been studied extensively.

The damage to red blood cells that is induced by 2-butoxyethanol appears to proceed through a process known as colloid osmotic haemolysis in which damage to the cell membrane leads to swelling of the red blood cell. In essence, the damage leads to a 'leaky' red cell or to other effects that produce a 'hole' in the red cell membrane that is smaller than the effective radius of the haemoglobin molecule. The extent of such damage governs the different processes that involve removal of damaged red blood cells from the circulation. Red blood cells that have minor damage and are slowly enlarging are removed totally in the spleen which has a particularly efficient mechanism to overcome stress and destroys subtly damaged red blood cells. More extensive damage can be recognized by the liver, which has a much larger capacity for removal of damaged red blood cells, but a higher threshold for recognition of damage. When the capacity of both spleen and liver are overwhelmed by a rapidly progressive swelling of the red blood cell, intravascular haemolysis occurs. Accordingly, in an individual with a spleen, low-level red cell damage does not

lead to the build-up of haemosiderin within the liver. It should be noted that some individuals are asplenic at birth or have their spleen removed due to trauma or to treatment for haematological or neoplastic disorders. In such individuals, senescent red blood cells are routinely removed in the liver, as would damaged red blood cells.

Chronic inhalation exposure to 2-butoxyethanol has been shown to result in the formation of hepatic haemangiosarcomas and hepatocellular carcinomas in male mice, but not in female mice or rats of either sex (National Toxicology Program, 2000; see Section 3). The induction of liver neoplasia has been suggested to be the result of oxidative damage secondary to the haemolytic deposition of iron in the liver (Park *et al*., 2002a; Sielsky *et al*., 2002; Klaunig & Kamendulis, 2005). It is postulated that the formation of liver tumours is mediated by the production of the haematotoxic metabolite of 2-butoxyethanol, 2-butoxyacetic acid, which causes haemolysis of red blood cells and the release of iron and its deposition in Kupffer cells in both rats and mice. These iron-containing deposits are assumed to catalyse, through Fenton and/or Haber-Weiss reactions, the formation of reactive oxygen species and/or to activate Kupffer cells to produce oxidative species in male mice as well as certain cytokines. The production of reactive oxygen species has been linked to the formation of oxidative DNA damage, i.e. 8-hydroxydeoxyguanosine, observed in hepatocytes (Park *et al.*, 2002a; Siesky *et al.*, 2002) and to the stimulation of DNA synthesis in both endothelial cells and hepatocytes (Siesky *et al.*, 2002) through a postulated modification of gene expression in the livers of male mice. It has been shown that induction of endothelial cell proliferation by the treatment of mice with 2-butoxyethanol occurs at doses that produced haemangiosarcomas in mice (Siesky *et al.*, 2002). The lack of tumour formation in rats has been attributed in part to a higher level of antioxidants, specifically vitamin E (2.5-fold greater), in rat liver than in mouse liver (Siesky *et al*., 2002). The more effective protection of rats against the consequences of oxidative stress is indicated by the lack of an increase in the formation of oxidative damage and the absence of enhanced proliferation of endothelial cells or hepatocytes, although haemolysis occurred (Siesky *et al*., 2002). In-vitro results have demonstrated that iron sulfate as well as iron released from haemolysed erythrocytes, but not 2-butoxyethanol, cause oxidative stress in primary cultured mouse hepatocytes and in Syrian hamster embryo cells, together with morphological transformation (Park *et al.*, 2002a,b), which adds support to the hypothesis that iron-induced oxidative stress may play an essential role in the induction of hepatic tumours by 2-butoxyethanol in male mice. Additional strong support for this hypothesis stems from the results of a search for a possible association between chemically induced haemosiderosis and haemangiosarcomas in the liver of mice in selected studies conducted by the National Toxicology Program (Nyska *et al.*, 2004). In addition to 2-butoxyethanol, two other compounds, i.e. *para*-nitroaniline and *para*-chloroaniline, were associated with haemosiderin pigmentation of Kupffer cells in both males and females and with the development of haemangiosarcoma in male mice only. The overall association between liver haemangiosarcoma and pigmentation in Kupffer cells was highly significant ($p < 0.001$). The cause of haemosiderosis in all cases was the haemolytic effect of the compounds (Nyska *et al*., 2004).

In considering all of the available evidence, a scenario appears conceivable whereby the exposure of male mice to high concentrations of 2-butoxyethanol results in haemolysis and iron-mediated oxidative stress in the liver, which is accompanied by the induction of mutagenic oxidative DNA lesions, such as 8-hydroxydeoxyguanosine, the repair of which by base-excision repair may be impeded by an interference of 2-butoxyethanol with poly-(ADP-ribosyl)ation. The simultaneously induced cell proliferation would increase the probability that DNA damage becomes a fixed mutation and that mutated clones expand and eventually give rise to tumours. However, other mechanisms have not been considered.

None of the known causes of hepatic haemangiosarcoma has been associated with haemolytic anaemia in humans. Similarly, no hepatic angiosarcoma has been reported in groups who have chronic haemolytic processes, such as the autoimmune haemolytic anaemia that is seen in patients who have lupus erythematosus, or individuals who have inherited red cell membrane defects, such as hereditary spherocytosis, for which splenectomy in childhood is the usual treatment. However, it should be noted that hepatic angiosarcomas are relatively rare and may not have been recognized. [The Working Group found no published studies on haemangiosarcomas associated with haemolysis or lupus.] Nevertheless, increased levels of iron in the body have been associated with other forms of cancer in the liver (Nyska *et al*., 2004).

Compared with that of mice and rats, the blood of humans is much more resistant to induction of haemolysis *in vitro* by 2-butoxyacetic acid. Moreover, the level of vitamin E in human liver is approximately 100-fold higher than that in mouse liver (Rocchi *et al*., 1997). Thus, it appears that the mechanism of oxidative stress is unlikely to occur in humans and that the hepatic tumours observed in male mice exposed to 2-butoxyethanol are a species- and sex-specific phenomenon that may not be relevant to humans.

Chronic inhalation exposure to 2-butoxyethanol has also been shown to be associated with the formation of forestomach tumours in female mice and, to a lesser extent, in male mice, whereas no forestomach tumours occurred in exposed rats. It has been proposed that tumour induction is the consequence of a sustained exposure of this organ to high concentrations of 2-butoxyethanol and its toxic metabolites, primarily 2-butoxyacetic acid, which results in tissue damage, reparative regeneration and hyperplasia (Green *et al*., 2002; Poet *et al*., 2003). Several factors are thought to contribute to a high local burden in the forestomach. These include direct exposure of the tissue, even in inhalation experiments, via the oral route as a consequence of grooming contaminated fur or licking the walls of the exposure chamber, salivary excretion and mucociliary transport of material deposited in the airways followed by ingestion (Green *et al*., 2002). In addition, other factors result in a prolonged exposure of the forestomach as compared with other organs. These include the physiological function of the forestomach as a storage organ for ingested material and the slow elimination of 2-butoxyethanol from forestomach tissue compared with other tissues or blood, irrespective of the route of application. The reason for the much lower susceptibility of rats to the development of forestomach lesions has not been elucidated fully. However, it has been shown in mice that ADH, which catalyses the first step in the oxidation of 2-butoxyethanol to 2-butoxyacetic acid, has a higher affinity and a higher maximal

activity with 2-butoxyethanol as substrate than rat ADH, and that it is expressed at much higher specific activity in areas of the mouse forestomach than in that of rats. These differences have been purported to result in higher local concentrations of toxic metabolites of 2-butoxyethanol in the forestomach of mice compared with that of rats (Green *et al.*, 2002).

It cannot be excluded that the continuous presence of the metabolite 2-butoxyacetaldehyde, which possibly exhibits weak clastogenic activity, can give rise to tumour formation in the regenerating forestomach tissue.

Anatomical and physiological differences between mice and humans limit, but do not entirely rule out, the relevance of mouse forestomach tumours to humans.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

2-Butoxyethanol is a glycol ether that is widely used as a solvent in surface coatings (paints and varnishes), paint thinners, printing inks and glass- and surface-cleaning products (including those used in the printing and silk-screening industries), and as a chemical intermediate. It is also used in a variety of personal care and other consumer products. Occupational exposure occurs through dermal absorption or via inhalation during its manufacture and use as a chemical intermediate, and during the formulation and use of its products. Highest mean exposures have been measured for silk screeners. Exposure of the general population can occur through dermal contact or inhalation during the use of consumer products, particularly cleaning agents.

5.2 Human carcinogenicity data

A case–control study of acute myeloid leukaemia and myelodysplasia found no elevation of risk with exposure to a group of glycol ethers, including 2-butoxyethanol. However, the information provided by this study on 2-butoxyethanol specifically was limited.

5.3 Animal carcinogenicity data

2-Butoxyethanol was tested for carcinogenicity by inhalation exposure in male and female mice and rats. Clear increases in tumour incidence were observed in a single species. Exposure to 2-butoxyethanol induced a dose-related increase in the incidence of haemangiosarcomas of the liver in male mice and a dose-related increase in the incidences of combined forestomach squamous-cell papillomas or carcinomas (mainly papillomas) in female mice. In female rats, a positive trend was observed in the occurrence of combined benign or malignant pheochromocytomas (mainly benign) of the