BENZYL ACETATE

Data were last reviewed in IARC (1986) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

1. Exposure Data

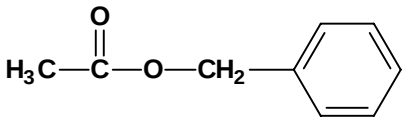
1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 140-11-4

Systematic name: Acetic acid, phenylmethyl ester

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_9H_{10}O_2$ Relative molecular mass: 150.18

1.1.3 *Physical properties* (for details, see IARC, 1986)

(*a*) *Boiling-point*: 215.5° C

(*b*) *Melting-point*: –51.3°C

(*c*) *Conversion factor*: $mg/m^3 = 6.14 \times ppm$

1.2 Occurrence, use and human exposure

Benzyl acetate has been identified in several fruits, such as bael fruit (from the *Aegle marmelos* tree) and quince (*Cydonia vulgaris*), and in a mushroom (*Agaricus* species). It is a major volatile constituent of the flowers of a number of plants, including jasmine (*Jasminium grandiflorum* L.), hyacinth (*Hyacinthus orientalis*), gardenia (*Gardenia jasminoides*), ylang-ylang (*Cananga odorata*), alfalfa (*Medicago sativa* L.) and others. It has been used as a food additive in fruit flavours and as a component of perfumes since the early 1990s and is widely used as a fragrance in soaps, detergents and incense. There is widespread human exposure to benzyl acetate by ingestion, skin application and inhalation.

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

Benzyl acetate was tested for carcinogenicity by gavage in one experiment in mice of both sexes and in one experiment in rats of both sexes. In the study in mice, increased incidences of liver adenomas and of combined liver adenomas and carcinomas were observed in animals of each sex; the incidence of carcinomas of the liver alone was not significantly increased in animals of either sex. An increased incidence of forestomach tumours was observed in mice of each sex. An increased incidence of acinar-cell adenomas of the pancreas was observed in male rats (IARC, 1986).

3.1 Oral administration

3.1.1 *Mouse*

Groups of 60 male and 60 female B6C3F_1 mice, six weeks of age, were administered benzyl acetate (purity, > 98%) in the diet at concentrations of 0, 330, 1000 or 3000 mg/kg diet (ppm) for two years. High-dose females had prolonged survival. The mean body weights of the two high-dose groups of both sexes were reduced. No increase in neoplasms was found in exposed mice (United States National Toxicology Program, 1993).

3.1.2 *Rat*

Groups of 49 or 38 male Fischer 344 rats, four weeks of age, were administered benzyl acetate [purity not specified] in the diet at 0 or 0.8%, respectively, for two years. Benzyl acetate had no significant adverse effect on growth or survival. The incidence of pancreatic adenomas was 10/49 (20%) in controls and 8/38 (21%) in the exposed group. Carcinoma *in situ* was found in 3/38 rats ($p = 0.0428$, chi-square test) compared to 0/49 controls [no other tissue was reported] (Longnecker *et al*., 1990).

Groups of 60 male and 60 female Fischer 344/N rats, six weeks of age, were administered benzyl acetate (purity, > 98%) in the diet at concentrations of 0, 3000, 6000 or 12 000 mg/kg diet (ppm) for two years. The mean body weights of both sexes given the high dose were slightly reduced. No increase in neoplasms was found in exposed rats (United States National Toxicology Program, 1993).

3.2 Administration with known carcinogens

Rat: Groups of four to five weanling male Lewis or Fischer 344 rats were administered daily 500 mg/kg bw benzyl acetate [purity unspecified] by gavage on five days per week or 0.9% in the diet for four months alone or following injection with 30 mg/kg bw azaserine at 14 days of age to initiate pancreatic carcinogenesis. Benzyl acetate alone

induced no pancreatic foci. When given after azaserine, no increase in pancreatic foci was produced (Longnecker *et al*., 1986).

Groups of 20 male Fischer 344 rats [age unspecified] were administered benzyl acetate [purity unspecified] at 0, 0.4 or 0.8% in the diet for 6–12 months either alone or following injection with azaserine to initiate pancreatic carcinogenesis. At six months, there were fewer pancreatic acidophilic foci in the groups fed benzyl acetate than in controls, but their mean diameter was increased. No effect on basophilic foci was found. In the groups observed for up to 12 months, survival was decreased in those given benzyl acetate. However, the incidence of carcinomas of the pancreas was decreased in a dose-related manner, i.e. 12% in controls, 8% in low-dose and 0% in high-dose rats (Longnecker *et al*., 1990).

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

4.1 Absorption, distribution, metabolism and excretion

The metabolism of benzyl acetate involves very rapid hydrolysis to acetate and benzyl alcohol. The latter is subsequently mainly oxidized to benzaldehyde and benzoic acid. A small part of the benzyl alcohol may be conjugated with sulfate, leading, ultimately, to formation of a glutathione conjugate that is excreted as mercapturate in urine. The benzoic acid is excreted mainly as hippurate and, to a lesser extent, as acyl glucuronide (see Figure 1).

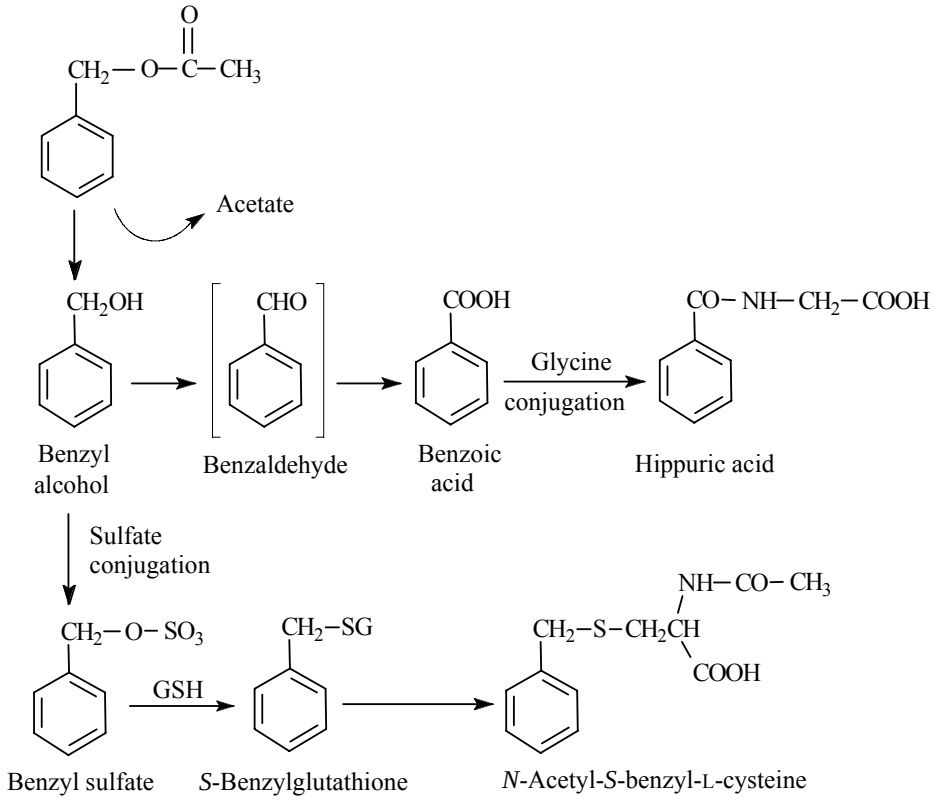
4.1.1 *Humans*

No recent data on humans are available, except for some very limited data on rate of skin penetration *in vitro* (see Section 4.1.2).

4.1.2 *Experimental systems*

Benzyl acetate is quite soluble in lipids and therefore readily absorbed from the gastrointestinal tract and lung, as well as through the skin, in the species investigated. The absorption after oral administration in the rat was delayed if it was administered in corn oil or propylene glycol as compared to neat [*methylene*-^{14}C]benzyl acetate (Chidgey & Caldwell, 1986): the peak plasma concentration of benzyl acetate-derived radioactivity occurred later (T_{max} after 1 h versus 4–6 h) and was lower at a 500 mg/kg benzyl acetate dose; at 5 mg/kg benzyl acetate, there was no difference. The urinary excretion of the metabolites was also delayed by corn oil, but the extent of absorption seemed not to be affected: more than 80% was absorbed and excreted within 24 h, mainly in urine and, ultimately, less than 5% in faeces. In plasma and urine, no intact benzyl acetate was detected at any time; only its metabolites were present (Chidgey & Caldwell, 1986). Benzyl acetate is rapidly hydrolysed by esterases to benzyl alcohol and acetate (Yuan *et al*., 1995). These esterases are present in plasma and probably also in the tissues; it is

Figure 1. Metabolism of benzyl acetate



Redrawn from Chidgey *et al.* (1986)

possible that during absorption benzyl acetate is already hydrolysed, so that little if any of the intact compound reaches the general circulation (Hotchkiss *et al*., 1992). The peak plasma levels of the metabolite benzoic acid in rats and mice fed benzyl acetate in the diet were much lower than after gavage of roughly the same dose, whereas the levels of hippurate were very similar (Yuan *et al*., 1995). The explanation may be that glycine conjugation of benzoic acid becomes saturated after the high, acute gavage dose, while during feeding the dose is more slowly taken up. Yuan *et al*. (1995) provide a pharmacokinetic model of benzyl acetate in rats and mice. Extensive absorption after dermal application of 100–500 mg/kg to rats was observed: 35–55% of the dose was recovered as metabolites in urine within 24 h (Chidgey *et al*., 1987). The metabolic profile was the same as after oral or intravenous administration. Some data on organ distribution of radioactivity were provided. This percutaneous absorption was confirmed *in vitro* with rat and human skin (Hotchkiss *et al*., 1990, 1992; Garnett *et al*., 1994). Dimethylsulfoxide had a minor enhancing effect on skin absorption. The absorption rate through rat skin was approximately

six-fold that through human skin. The identity of the absorbed radioactivity was not determined, but most likely represented benzyl acetate metabolites.

No unchanged benzyl acetate was detected in rat or mouse plasma. The plasma concentrations of metabolites after intravenous administration of 5 mg/kg bw benzyl acetate (McMahon *et al*., 1989) showed only minor changes with age (3–4, 9 and 25 months) in Fischer 344 rats and $B6C3F_1$ or C57BL/6N mice. In urine, by far the major metabolite is the hippurate at all doses and in all species (Abdo *et al*., 1985; Chidgey & Caldwell, 1986; McMahon *et al*., 1989). The acyl glucuronide of benzoic acid comprised from 2 to 12% of the urinary metabolites (low versus high dose) and benzyl mercapturate 1–2% of the dose (Chidgey & Caldwell, 1986). This mercapturate is probably formed from benzyl alcohol through its (reactive) sulfate ester, since prevention of oxidation by pyrazole increases the mercapturate from 1.1 to 12% of the dose, while the sulfation inhibitor pentachlorophenol subsequently decreases it again to 2% (Chidgey *et al*., 1986).

4.1.3 *Comparison of human and rodent data*

Since no data are available on humans except for skin penetration data *in vitro*, no direct comparison can be made. Because in humans most of the dose seems to be excreted as hippurate (IARC, 1986) and because the metabolism of the primary hydrolysis product of benzyl acetate, benzyl alcohol, is very similar in rodents and humans (see, e.g., the monograph on toluene (this volume)), it is to be expected that the fate of benzyl acetate in humans is very similar to that in rodents.

4.2 Toxic effects

4.2.1 *Humans*

No data were available to the Working Group.

4.2.2 *Experimental systems*

In a study in male and female mice, the survival of the animals was not decreased at any dose level (IARC, 1986).

4.3 Reproductive and developmental effects

No data were available to the Working Group.

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 1 for references)

Benzyl acetate gave negative results in the *Bacillus subtilis rec* assay. It was not mutagenic to *Salmonella typhimurium* in the presence or absence of exogenous metabolic activation nor did it induce sex-linked recessive lethal mutations in *Drosophila melanogaster* following exposure by injection.

Table 1. Genetic and related effects of benzyl acetate

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
BSD, *Bacillus subtilis rec*, differential toxicity	–	NT	21	Oda *et al*. (1978)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	450	Florin *et al*. (1980)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	1667	Mortelmans *et al*. (1986)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	450	Florin *et al*. (1980)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	1667	Mortelmans *et al*. (1986)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	450	Florin *et al*. (1980)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	500	Mortelmans *et al*. (1986)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	450	Florin *et al*. (1980)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	5000	Mortelmans *et al*. (1986)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	–		20000 inj	US National Toxicology Program (1993)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	+	NT	900	McGregor *et al*. (1988)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	–	+	750	Caspary *et al*. (1988)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	–	–	5000	Galloway *et al*. (1987)
CIC, Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	–	–	5000	Galloway *et al*. (1987)
CIC, Chromosomal aberrations, Chinese hamster lung CHL fibroblasts *in vitro*	–	–	300	Matsuoka *et al*. (1996)
AIA, Aneuploidy, Chinese hamster lung CHL fibroblasts *in vitro*	–	–	300	Matsuoka *et al*. (1996)
DNA strand breaks and related damage (alkaline elution assay) in rat pancreas *in vivo*	–		1500 ip × 1	Longnecker *et al*. (1990)
UPR, Unscheduled DNA synthesis, Fischer 344 rat hepatocytes *in vivo*	–		1000 po × 1	Mirsalis *et al*. (1989)

Table 1 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
SVA, Sister chromatid exchange, B6C3F$_1$ mouse bone marrow *in vivo*	–		1700 ip × 1	US National Toxicology Program (1993)
MVM, Micronucleus test, B6C3F$_1$ mouse bone marrow *in vivo*	–		1250 ip × 3	US National Toxicology Program (1993)
MVM, Micronucleus test, B6C3F$_1$ mouse peripheral blood lymphocytes *in vivo*	–		6000 feed 13 wk	US National Toxicology Program (1993)
CBA, Chromosomal aberrations, B6C3F$_1$ mouse bone marrow *in vivo*	–		1700 ip × 1	US National Toxicology Program (1993)

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

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

Benzyl acetate did not induce unscheduled DNA synthesis in rat primary hepatocytes *in vitro* in a single study, as reported in an abstract (Mirsalis *et al.*, 1983). It was mutagenic in mouse lymphoma L5178Y cells at the *tk* locus with or without the addition of exogenous metabolic activation. It did not induce sister chromatid exchanges or chromosomal aberrations in Chinese hamster ovary cells nor chromosomal aberrations or aneuploidy in Chinese hamster lung fibroblasts in the presence or absence of exogenous metabolic activation.

Benzyl acetate did not induce unscheduled DNA synthesis in hepatocytes of rats following gavage treatment and a single intraperitoneal dose did not induce DNA damage in the pancreas of rats. It did not increase the frequency of sister chromatid exchanges, chromosomal aberrations or micronuclei in the bone marrow of $B6C3F_1$ mice *in vivo*, nor did it induce micronuclei in peripheral blood lymphocytes of mice given benzyl acetate for 13 weeks in their diet.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

There is widespread human exposure to benzyl acetate by ingestion, skin application and inhalation.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

Benzyl acetate was tested for carcinogenicity by gavage in one experiment in mice and in one experiment in rats, and by administration in the diet in two studies in rats and in one study in mice. In the gavage study in mice, increased incidences of liver adenomas and of combined liver adenomas and carcinomas were oberved in animals of each sex. An increased incidence of forestomach tumours was observed in mice of each sex. An increased incidence of acinar-cell adenomas of the pancreas was observed in male rats administered benzyl acetate by gavage. Benzyl acetate did not increase the incidence of tumours in either mice or rats when administered in the diet. A low incidence of pancreatic carcinomas *in situ* was reported in one study.

Benzyl acetate was tested in two studies for promotion of pancreatic carcinogenesis in rats and was found to be inactive.

5.4 Other relevant data

Benzyl acetate is hydrolysed to benzoic acid and acetate. It is metabolized similarly by humans and rodents. Except for one positive result *in vitro*, findings on genotoxicity *in vitro* and *in vivo* were negative.

5.5 Evaluation

No epidemiological data relevant to the carcinogenicity of benzyl acetate were available.

There is *limited evidence* in experimental animals for the carcinogenicity of benzyl acetate.

Overall evaluation

Benzyl acetate is *not classifiable as to its carcinogenicity to humans (Group 3)*.

6. References

Abdo, K.M., Huff, J.E., Haseman, J.K., Boorman, G.A., Eustis, S.L., Matthews, H.B., Burka, L.T., Prejean, J.D. & Thompson, R.B. (1985) Benzyl acetate carcinogenicity, metabolism, and disposition in Fischer 344 rats and B6C3F_1 mice. *Toxicology*, **37**, 159–170

Caspary, W.J., Langenbach, R., Penman, B.W., Crespi, C., Myhr, B.C. & Mitchell, A.D. (1988) The mutagenic activity of selected compounds at the TK locus: rodent vs. human cells. *Mutat. Res.*, **196**, 61–81

Chidgey, M.A.J. & Caldwell, J. (1986) Studies on benzyl acetate. I. Effect of dose size and vehicle on the plasma pharmacokinetics and metabolism of [*methylene*-^{14}C]benzyl acetate in the rat. *Food chem. Toxicol.*, **24**, 1257–1265

Chidgey, M.A.J., Kennedy, J.F. & Caldwell, J. (1986) Studies on benzyl acetate. II. Use of specific metabolic inhibitors to define the pathway leading to the formation of benzylmercapturic acid in the rat. *Food chem. Toxicol.*, **24**, 1267–1272

Chidgey, M.A.J., Kennedy, J.F. & Caldwell, J. (1987) Studies on benzyl acetate. III. The percutaneous absorption and disposition of [*methylene*-^{14}C]benzyl acetate in the rat. *Food chem. Toxicol.*, **25**, 521–525

Florin, I., Rutberg, L., Curvall, M. & Enzell, C.R. (1980) Screening of tobacco smoke constituents for mutagenicity using the Ames' test. *Toxicology*, **18**, 219–232

Galloway, S.M., Armstrong, M.J., Reuben, C., Colman, S., Brown, B., Cannon, C., Bloom, A.D., Nakamura, F., Ahmed, M., Duk, S., Rimpo, J., Margolin, B.H., Resnick, M.A., Anderson, B. & Zeiger, E. (1987) Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: evaluations of 108 chemicals. *Environ. mol. Mutag.*, **10** (Suppl. 10), 1–175

Garnett, A., Hotchkiss, S.A.M. & Caldwell, J. (1994) Percutaneous absorption of benzyl acetate through rat skin *in vitro*. 3. Comparison with human skin. *Food chem. Toxicol.*, **32**, 1061–1065

Hotchkiss, S.A., Chidgey, M.A., Rose, S. & Caldwell, J. (1990) Percutaneous absorption of benzyl acetate through rat skin *in vitro*. 1. Validation of an in vitro model against in vivo data. *Food chem. Toxicol.*, **28**, 443–447

Hotchkiss, S.A.M., Miller, J.M. & Caldwell, J. (1992) Percutaneous absorption of benzyl acetate through rat skin *in vitro*. 2. Effect of vehicle and occlusion. *Food chem. Toxicol.*, **30**, 145–153

IARC (1986) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Vol. 40, *Some Naturally Occurring and Synthetic Food Components, Furocoumarins and Ultraviolet Radiation*, Lyon, pp. 109–122

IARC (1987) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Supplement 7, *Overall Evaluations of Carcinogenicity: An Updating of* IARC Monographs *Volumes 1 to 42*, Lyon, p. 58

Longnecker, D.S., Roebuck, B.D., Curphey, T.J., Lhoste, E., Coon, C.I. & MacMilan, D. (1986) Effects of corn oil and benzyl acetate on number and size of azaserine-induced foci in the pancreas of LEW and F344 rats. *Environ. Health Perspect.,* **68**, 197–201

Longnecker, D.S., Roebuck, B.D., Curphey, T.J. & MacMillan, D.L. (1990) Evaluation of promotion of pancreatic carcinogenesis in rats by benzyl acetate. *Food chem. Toxicol.,* **28**, 665–668

Matsuoka, A., Yamakage, K., Kusakabe, H., Wakuri, S., Asakura, M., Noguchi, T., Sugiyama, T., Shimada, H., Nakayama, S., Kasahara, Y., Takahashi, Y., Miura, K.F., Hatanaka, M., Ishidate, M., Jr, Morita, T., Watanabe, K., Hara, M., Odawara, K., Tanaka, N., Hayashi, M. & Sofuni, T. (1996) Re-evaluation of chromosomal aberration induction on nine mouse lymphoma assay 'unique positive' NTP carcinogens. *Mutat. Res.*, **369**, 243–252

McGregor, D.B., Brown, A., Cattanach, P., Edwards, I., McBride, D., Riach, C. & Caspary, W.J. (1988) Responses of the L5178Y tk^+/tk^- mouse lymphoma cell forward mutation assay: III. 72 coded chemicals. *Environ. mol. Mutag.*, **12**, 85–154

McMahon, T.F., Diliberto, J.J. & Birnbaum, L.S. (1989) Age-related changes in the disposition of benzyl acetate. A model compound for glycine conjugation. *Drug Metab. Disp*., **17**, 506–512

Mirsalis, J., Tyson, K., Beck, J., Loh, F., Steinmetz, K., Contreras, C., Austere, L., Martin, S. & Spaulding, J. (1983) Induction of unscheduled DNA synthesis (UDS) in hepatocytes following in vitro and in vivo treatment (Abstract). *Environ. Mutag.*, **5**, 482

Mirsalis, J.C., Tyson, C.K., Steinmetz, K.L., Loh, E.K., Hamilton, C.M., Bakke, J.P. & Spalding, J.W. (1989) Measurement of unscheduled DNA synthesis and S-phase synthesis in rodent hepatocytes following in vivo treatment: testing of 24 compounds. *Environ. mol. Mutag.*, **14**, 155–164

Mortelmans, K., Haworth, S., Lawlor, T., Speck, W., Tainer, B. & Zeiger, E. (1986) *Salmonella* mutagenicity tests: II. Results from the testing of 270 chemicals. *Environ. Mutag.*, **8** (Suppl. 7), 1–119

Oda, Y., Hamano, Y., Inoue, K., Yamamoto, H., Niihara, T. & Kunita, N. (1978) Mutagenicity of food flavors in bacteria (1st report). *Osaka Pref. Inst. Publ. Health, ED Food Microbiol.*, **9**, 177–181 (in Japanese)

United States National Toxicology Program (1993) *Toxicology and Carcinogenesis Studies of Benzyl Acetate (CAS No. 140-11-4) in F344/N Rats and B6C3F$_1$ Mice* (NTP Technical Report Series No. 431; NIH Publ. No. 93-3162), Research Triangle Park, NC

Yuan, J.H., Goehl, T.J., Abdo, K., Clark, J., Espinosa, O., Bugge, C. & Garcia, D. (1995) Effects of gavage versus dosed feed administration on the toxicokinetics of benzyl acetate in rats and mice. *Food chem. Toxicol.*, **33**, 151–158