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Opinion
of the Scientific Committee on Food
on Benzoic acid and its salts

(expressed on 24 September 2002)

Opinion on Benzoic acid and its salts

Terms of reference

The Committee is asked to re-evaluate its earlier opinion of 1994 on the safety of benzoic acid and its salts as food additives in the light of new information.

Background

Benzoic acid and its salts are in widespread use as food preservatives in the EU. The Scientific Committee for Food (SCF) was first asked to evaluate the safety of benzoic acid and its salts in 1994. In this opinion (SCF, 1994), the Committee raised questions about developmental toxicity and genotoxicity and asked for further studies in these two areas. In view of these data requests, the Committee set only a temporary ADI, of 0 - 5 mg/kg bw based on an overall NOAEL of 500 mg/kg bw/day from long-term and multigeneration studies.

The benzoates had previously been evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), which established an ADI of 0 - 5 mg/kg bw as a sum of benzoic acid and its salts, expressed as benzoic acid (JECFA, 1974). At a later meeting, JECFA recommended a full review of benzoic acid and its salts, together with the flavouring substances benzyl acetate, benzyl alcohol and benzaldehyde (JECFA 1993), in order to determine whether additional studies were needed. In particular the absence of reproductive and developmental toxicity studies was noted. These substances were grouped because of their common metabolic pathway. After receipt of new information, they were re-evaluated in 1996 and the Group ADI of 0 - 5 mg/kg bw was maintained (JECFA, 1997). JECFA was satisfied that the data were sufficient to demonstrate lack of carcinogenicity and lack of toxicity to development and reproduction. The genotoxicity database was also reviewed at that time.

In response to several requests from the Commission for further information addressing the issues raised by the SCF in 1994, industry submitted a commentary prepared by an outside expert (ELC, 2001).

Developmental toxicity issues

SCF 1994 evaluation

In relation to developmental toxicity, the Committee noted in 1994 that hippuric acid, the glycine conjugate of benzoic acid, was the main urinary metabolite and there was evidence in both humans and rodents that large, bolus doses can cause glycine depletion. The Committee's conclusion (SCF, 1994) was as follows:

“The data available give adequate reassurance that the use of benzoic acid and its salts as food preservatives is temporarily acceptable. However, the role of glycine

in the rate limiting step for hippuric acid formation from benzoic acid suggests that there may be a narrow margin between the metabolic demand for glycine and the rate at which glycine is formed or made available in the body. Glycine is not generally regarded as an essential amino acid but it has been suggested that in rapidly growing organisms glycine may be a conditionally essential amino acid and that this fine balance might be disturbed by benzoic acid. An adequate teratogenicity study using a dietary route of administration is therefore desirable.”

The only studies considered by the SCF in reaching its 1994 opinion were a four-generation study on benzoic acid (Kieckebusch and Lang, 1960) and an intraperitoneal developmental toxicity study on sodium benzoate (Minor and Becker, 1971). The latter study was regarded by the SCF as inadequate for evaluation of dietary benzoate due to the route of administration. Other studies, that have since been reviewed by JECFA (1997), were completed by the time the SCF concluded its 1994 opinion, but several were unpublished reports and were not available to the SCF at that time.

Industry commentary

The commentary submitted by industry (ELC, 2001) agreed that the main transformation pathway for benzoic acid to hippuric acid involves a saturable process at high doses in both humans and most experimental species, in which the availability of glycine is the rate-limiting step. However, it went on to say that whereas this conclusion as such may be valid, the following points should be taken into account:

1. The likelihood of glycine depletion is strongly dependent on the (high) dose of benzoic acid to which the body is exposed.
2. A number of reproduction studies including teratogenicity studies have been carried out by oral route with benzoic acid, its salt and other members of the group of benzyl compounds.

Of the now available studies on reproduction and developmental toxicity, all except one of those cited in the industry submission (ELC, 2001) have been fully described in the JECFA Monograph (JECFA, 1997). Based on the results of these studies, the industry submission concluded that it was unlikely that new teratogenicity studies on benzoic acid via the diet would lead to results that would affect the established ADI.

Re-evaluation

Given the ample evidence of a common and rapid route of metabolism of the salts of benzoic acid and the three benzyl flavourings to benzoic acid and subsequently to hippuric acid (JECFA, 1997), it is reasonable to assume that studies on any of these substances provide valid information for the assessment of the toxicity of benzoic acid and its salts. On this basis, there are three reproduction studies and three developmental toxicity studies that are relevant to the issue raised by the SCF in 1994. A summary of these studies is attached as Annex 1.

In the reproduction studies, the multigeneration study in rats using dietary administration of benzoic acid found no effects on birth weight, postnatal growth or survival up to 750 mg/kg bw/day (Kieckebusch and Lang, 1960). In the mouse gavage studies on benzyl alcohol, a lowest-observed-adverse-effect level (LOAEL) of 750 mg/kg bw/day for effects on pup weight and a no-observed-adverse-effect level (NOAEL) of 550 mg/kg bw/day were identified (York et al., 1986).

In the developmental toxicity studies, fetotoxic effects were described in one gavage study on rats following benzyl acetate at 1000 mg/kg bw/day, with a NOAEL of 500 mg/kg bw/day (Ishiguro et al., 1993). In the gavage studies on sodium benzoate in rats, mice, hamsters and rabbits, the top doses used (175-300 mg/kg bw/day) were somewhat low but were without adverse effects (Food and Drug research Labs, Inc., 1972). In a dietary study on sodium benzoate, adverse effects on the fetuses and delivered offspring were seen at very high doses, but a NOAEL of 1310 mg/kg bw/day was identified (Onodera et al., 1978).

Since benzoic acid is readily absorbed and metabolised, if adverse effects due to depletion of glycine availability for metabolism were to occur, it is likely they would be most evident in oral studies using the gavage route, delivering bolus doses, rather than in studies using the dietary route, in which peak blood levels of parent compound would be many-fold lower (Yuan et al., 1995). It appears to be the case that NOAELs from gavage administration were slightly lower than those from dietary administration. The exact mechanism of the fetal and offspring toxicity, seen at high doses in some studies, cannot be determined from the data available; it could be secondary to maternal toxicity. However, identifying the mechanism of toxicity is not critical to the evaluation since there are adequate data to establish an overall NOAEL of 500 mg/kg bw/day.

Genotoxicity issues

SCF 1994 evaluation

In relation to genotoxicity, the Committee's conclusion in 1994 (SCF, 1994) was as follows:

“The observations of clastogenic activity of benzoic acid *in vitro* indicate that it should be tested for clastogenic activity *in vivo* in peripheral lymphocytes or bone marrow in animals and that blood or bone marrow levels respectively of benzoic acid should be measured in such a study”.

In reaching its 1994 opinion the SCF did not consider several studies, including most of the *in vivo* ones, cited by JECFA (1997) or in the industry submission (ELC, 2001). Several of them were not available at that time.

Industry commentary

The commentary submitted by industry stated that since benzylacetate, benzylalcohol and benzylaldehyde are all metabolised to benzoic acid, the *in vivo* genotoxicity tests performed with these substances and on sodium benzoate may be applied also to benzoic

acid. Based on the negative results obtained in all *in vivo* genotoxicity studies available on sodium benzoate and the other benzyl compounds it was concluded that the need for an *in vivo* study to investigate the clastogenic activity of benzoic acid was not justified.

Re-evaluation

In view of the additional genotoxicity data now available, the Committee has compiled its own summary of the studies, attached as Annex 2. The Committee agrees that since benzylacetate, benzylalcohol and benzylaldehyde are all metabolised to benzoic acid, the results of *in vivo* tests performed with these compounds as well as with sodium benzoate may be applied also to benzoic acid itself. Considering the database as a whole, weak genotoxic effects have been reported mainly at chromosome level in some *in vitro* systems. However, all the *in vivo* genotoxicity tests were negative at somatic or germ cell level. The essentially negative results obtained in three carcinogenicity studies (one in mice, two in rats) on sodium benzoate, notwithstanding some limitations, give further reassurance. On this basis, it is very unlikely that benzoic acid would interfere with chromosomes *in vivo*.

Conclusions

The database is much more extensive than that considered by the Committee in 1994, both for developmental toxicity and for genotoxicity.

There appear to be sufficient studies to conclude absence of teratogenic potential, with an overall NOAEL for developmental toxicity of 500 mg/kg bw/day, based on effects on fetal weight. The fact that this overall NOAEL takes into account gavage as well as dietary studies gives further reassurance. It is therefore concluded that a further teratogenicity study on benzoic acid should no longer be required.

Similarly for genotoxicity, while some of the *in vitro* tests have been positive or equivocal, all the results from *in vivo* studies have been negative. It is therefore concluded that an *in vivo* study for clastogenic activity on benzoic acid should no longer be required.

On the basis of these data and the other types of study previously evaluated by the Committee, the Committee can establish a full Group ADI of 0 - 5 mg/kg bw for benzoic acid and its salts including benzyl alcohol and related benzyl derivatives used as flavourings.

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Summary of reproduction/developmental toxicity studies on benzoic acid and related compounds

Benzyl alcohol

In two separate studies in mice, benzyl alcohol was given by gavage to 50 animals with 50 vehicle-treated controls, at a single dose of either 550 mg/kg bw/day on days 6-15 of gestation (York et al., 1986) or 750 mg/kg bw/day on days 7-14 of gestation (Hardin et al., 1987; also quoted as US NIOSH, 1983). All dams were allowed to litter out and observed until 3 days *post-partum*. In the first study, there were no effects from 550 mg/kg bw/day on litter size, maternal or offspring mortality or body weights. In the second study there were no effects on litter size or pup survival, but there were 18 maternal deaths with clear clinical signs of toxicity in most survivors, reduced maternal weight gain, and reduced pup birth weight and postnatal weight gain.

Benzyl acetate

In a developmental toxicity study in rats, benzyl acetate was given by gavage to groups of 20 rats at 0, 10, 100, 500 or 1000 mg/kg bw/day on days 6-15 of gestation (Ishiguro et al., 1993). There were no teratogenic effects. On the basis of fetotoxic effects at 1000 mg/kg bw/day, comprising reduced fetal weight and increases in dilated renal pelvis, skeletal variations and retarded ossification, a NOAEL of 500 mg/kg bw/day could be established.

Sodium benzoate

In a rat developmental toxicity study, sodium benzoate was given by intraperitoneal injection at doses of 0, 100, 315 or 1000 mg/kg bw/day on days 9-11 or 12-14 of gestation (Minor and Becker, 1971). In this study, fetal death and reduced fetal weight (both time periods) and an increase in unspecified gross malformations (days 9-11 only) were observed at 1000 mg/kg bw/day. This study was published in abstract only, and did not contain any statistical evaluations.

In developmental toxicity studies, four doses of sodium benzoate were given by gavage during the relevant periods of organogenesis to groups of 20 or 24 mice, rats or golden hamsters or groups of 10 Dutch belted rabbits (Food and Drug Research Labs, Inc. 1972). The highest doses tested were 175, 175, 300 and 250 mg/kg bw/day respectively in mice rats, hamsters and rabbits. There were both positive and negative control groups for each species. No adverse maternal effects or effects on the embryos or fetuses were found.

In another developmental toxicity study, sodium benzoate was administered to groups of 27-30 rats at levels of 0, 1, 2, 4 or 8% in the diet (Onodera et al., 1978). All except 5 dams were sacrificed on day 20 of gestation. The remainder were allowed to litter out and rear their pups to weaning. Some pups were killed at weaning and others at 8 weeks of age. At 4% in the diet there was a significant reduction in maternal weight gain and at 8% there was maternal weight loss, with reduced food intake in both groups. At 4 and 8%, there was increased embryofetal mortality, reduced fetal weight and increases in gross,

soft tissue and skeletal abnormalities and anomalies. In those allowed to litter out there was reduced delivery rates and reduced postnatal survival. At 1 and 2% in the diet (700 mg/kg bw/day and 1310 mg/kg bw/day respectively) no adverse effects were seen.

Benzoic acid

Benzoic acid was given by gavage to 7 Wistar rats on day 9 of gestation at a dose of 510 mg/kg bw/day (Kimmel et al., 1971). There were 6 controls. Implantations, resorption sites and live foetuses were counted and the foetuses weighed and examined for gross malformations and by histopathological examination. Benzoic acid did not influence implantation, was not teratogenic, and did not lead to death or resorption. This study was not discussed by JECFA (1997) and is inadequate for evaluation due to the single day of compound administration.

Benzoic acid administered to rats in the diet at levels of 0.5 or 1% (approximately 375 and 750 mg/kg bw /day) for four generations did not affect growth, protein efficiency or reproduction. Survival of the first generation was significantly better for the 0.5% treated rats, and showed a non-significant increase at the 1% level (Kieckebusch and Lang, 1960).

ANNEX 2

Summary of genotoxicity studies on benzoic acid and related compounds

Benzoic acid

Benzoic acid was unable to induce mutations in *Salmonella typhimurium* and mitotic recombination in *Saccharomyces cerevisiae* (Brusick, 1975; Ishidate et al., 1984; Zeiger et al., 1988). Equivocal results were obtained in a chromosomal aberration test in cultured Chinese hamster fibroblast cell line (1.5 mg/ml) (Ishidate et al., 1984). It was negative in three *in vitro* sister chromatid exchange (SCE) tests (Oikawa et al., 1980; Tohda et al., 1980; Jansson et al., 1988).

Sodium benzoate

Sodium benzoate was negative in the *S.typhimurium* reverse mutation assay (Ishidate et al., 1984). It induced chromosomal aberrations in cultured Chinese hamster lung cells (up to 2000µg/ml). (Ishidate et al., 1984). It induced several cytological effects and micronuclei in *Vicia faba* (Njagi and Gopalan, 1982). It was negative in an *in vitro* chromosome aberration test at levels up to 200 µg/ml and in three *in vivo* assays (Litton Bionetics, 1974): (a) a host mediated assay after single or multiple dosing of ICR mice at levels 50, 500 and 5000 mg/kg bw/day by gavage was carried out using *S.typhimurium* G46, *S.typhimurium* TA1530 and *S. cerevisiae* D3. With the exception of elevated mutant frequency at the intermediate single dose level for strain TA1530, all other strains and single or multiple doses gave negative results; (b) a chromosomal aberration assay was carried out in bone marrow cells of Sprague-Dawley rats exposed by gavage to single or repeated doses for 5 consecutive days of 50, 500 and 5000 mg/kg bw/day sodium benzoate, with negative results; (c) a dominant lethal test using 50, 500 or 5000 mg/kg bw/day as a single or multiple (5 days) doses to male Sprague-Dawley rats was negative.

Benzyl acetate

Benzyl acetate was negative in the reverse mutation assay in *S.typhimurium* and positive, with and without S9, in the mouse lymphoma tk assay (US NTP, 1993; Caspary et al., 1988; McGregor et al., 1988). It gave equivocal results in the chromosomal aberration assay and negative results in the SCE assay in cultured Chinese hamster ovary (CHO) cells (US NTP, 1993; Galloway et al., 1987), It induced mitotic chromosome loss in *S. cerevisiae* (Zimmerman et al., 1989). It was negative in three different *in vitro* unscheduled DNA synthesis (UDS) tests (Mirsalis et al., 1989; Steinmetz and Mirsalis, 1984). It was negative in five *in vivo* genotoxicity assays carried out by the US NTP (1993): a sex-linked recessive lethal mutation assay in *Drosophila melanogaster*; a chromosomal aberration assay in bone marrow cells of mice treated i.p. (325-1700 mg/kg bw); two mouse bone marrow micronucleus assays (one by i.p. at 312-1250 mg/kg bw and one in diet at 3130-50000 ppm); a SCE in bone marrow cells of mice treated i.p. (325-1700 mg/kg bw). Another sex-linked recessive lethal mutation assay in *D. melanogaster*, where benzyl acetate was fed or injected, gave negative results (Fouremant, 1994). It was weakly positive in an assay on replicative DNA synthesis in B6C3F₁ mice only at the highest dose (1600 mg/kg bw/day) (Miyagawa et al., 1995).

Benzyl alcohol

Benzyl alcohol was negative in the in the reverse mutation assay in *S.typhimurium* in several studies (Florin et al., 1980; Wissler et al., 1983; Ishidate, 1984; Mortelmans et al., 1986; US.NTP, 1989). It was positive in the mouse lymphoma *tk* assay only without metabolic activation (McGregor et al., 1988; US NTP, 1989). It induced chromosomal aberrations in CHO cells without S9 and was weakly positive with S9 (US NTP, 1989), while Anderson et al. (1990) observed positive results only with S9 at high concentrations. A negative result from an *in vitro* chromosomal aberration assay was reported by Ishidate et al. (1984). The induction of SCEs in CHO cells was found to be equivocal (US NTP, 1989; Anderson et al., 1990). In an *in vitro* alkaline elution/rat hepatocytes assay it produced DNA double-strand breaks at cytotoxic concentrations (Storer et al., 1996). *In vivo*, it was negative in the micronucleus assay on mouse bone marrow cells (Hayashi et al., 1988), in an assay on replicative DNA synthesis in rats (Uno et al., 1994; Miyagawa et al., 1995), and in the sex-linked recessive lethal assay in *D.melanogaster* (Foureman et al., 1994).

Benzaldehyde

Benzaldehyde was negative in the reverse mutation assay in *S.typhimurium* in several studies (Florin et al., 1980; Kasamaki et al., 1982; Haworth et al., 1983; Wiessler et al., 1983; US NTP, 1990). It was positive in the mouse lymphoma *tk* assay (US NTP, 1990; McGregor et al., 1991). Positive results (up to 50 nmol/l) (Kasamaki et al., 1982) and negative results (up to 1600 µg/ml) (Galloway et al., 1987; US NTP, 1990) were obtained in an *in vitro* chromosomal aberration assay in CHO cells. Positive results were obtained in a SCE assay in cultured human lymphocytes (Jansson et al., 1988). It was negative in the sex-linked recessive lethal mutation assay in *D.melanogaster* (Woodruff et al., 1985; US NTP, 1990).

Hippuric acid

Hippuric acid was negative in a reverse mutation assay in *S.typhimurium* (Wiessler et al., 1983).