Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on a Request from the Commission related to para hydroxybenzoates (E 214-219)

Question number EFSA-Q-2004-063

(adopted on 13 July 2004)

SUMMARY

The Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food has been asked to provide an opinion on the safety of paraben (E 214-219) usage in foods by 1 July 2004.

The EC Scientific Committee for Food (SCF) evaluated the parabens in 1994 and established a temporary Acceptable Daily Intake (ADI) of 0-10 mg/kg bw, as the sum of methyl, ethyl and propyl p-hydroxybenzoic acid esters and their sodium salts. The temporary ADI was based on long-term studies in rats with methyl, ethyl and propyl paraben. The ADI was made temporary because the SCF considered that the toxicological information available showed some inadequacies and uncertainties. The SCF therefore requested a new oral teratogenicity study in the rat using either free p-hydroxybenzoic acid or its methyl, ethyl or propyl ester and a cell proliferation study in the rat on the propyl ester of p-hydroxybenzoic acid given as a solution. In 2000, the SCF reiterated its wish to review the safety of parabens and at its last meeting in April 2003 the SCF noted that no data had been submitted by the food industry in support of the parabens and drew attention to its statement of October 2000, that the temporary ADI should be withdrawn if no further data were submitted.

The SCF had previously requested an oral teratogenicity study in the rat. The Panel evaluated newly available studies on the developmental toxicity of methyl paraben in rats, mice, hamsters, and rabbits which were not available to the SCF when it made its request for a teratogenicity study. No evidence of developmental toxicity up to and including the highest

tested doses of 300 (rabbits) or 550 mg/kg body weight/day (rodents) were observed. The Panel concluded that no further data on developmental toxicity were needed.

The Panel also re-evaluated the proliferative effects of parabens on forestomach cells in rats, and concluded that the proliferative effect of parabens will only occur above a certain threshold and that the human exposure resulting from the use of parabens as preservatives in food will be much below such doses.

Consequently the Panel considered that the study previously requested by the SCF on cell proliferation in the rat on the propyl ester of p-hydroxybenzoic acid given as a solution were no longer needed.

Several parabens have shown oestrogenic activity *in vitro*. However, no oestrogenic activity could be detected *in vivo* for methyl, ethyl, and propyl parabens in classical uterotrophic assays using peroral or subcutaneous administrations of high doses to mice and rats. An *in vivo* uterotrophic effect was observed after subcutaneous injection of either butyl paraben or isobutyl paraben, which are not used as food additives. The common metabolite of parabens, p-hydroxybenzoic acid, was considered to be non-oestrogenic.

Dietary administration of propyl paraben to juvenile male rat for four weeks was reported to reduce the daily sperm production in the testis in all dose groups, including the lowest dose levels of 10 mg /kg body weight/day. At higher dose levels, reduced numbers of sperm cells, impaired spermatogenesis, and reduced testosterone levels were also observed. Thus, 10 mg/kg body weight/day was considered a Lowest Observed Adverse Effect Level (LOAEL) for propyl paraben. In contrast, methyl and ethyl paraben showed no effects on sex hormones and the male reproductive organs in juvenile rats at dose levels up to 1000 mg/kg body weight/day. Therefore 1000 mg/kg body weight/day was considered a No Observed Adverse Effect Level (NOAEL) for both methyl paraben and ethyl paraben.

The Panel established a full group ADI of 0-10 mg/kg bw for the sum of methyl and ethyl p-hydroxybenzoic acid esters and their sodium salts on the basis of the NOAELs of 1000 mg/kg bw/day for each compound in long-term toxicity studies and studies on sex hormones and the male reproductive organs in juvenile rats. The Panel considered that propyl paraben should

not be included in this group ADI because propyl paraben, contrary to methyl and ethyl paraben, had effects on sex hormones and the male reproductive organs in juvenile rats.

The Panel is unable to recommend an ADI for propyl paraben because of the lack of a clear NOAEL.

KEY WORDS

para-Hydroxybenzoic acid alkyl esters, parabens, food additive, preservative

BACKGROUND

P-hydroxybenzoic acid alkyl esters, "parabens", are antimicrobial preservatives allowed for use in foods, drugs, cosmetics and toiletries. They are normally used in combinations containing two or more parabens and/or other preservatives. Under EC Directive 95/2/EC, Annex III, methyl-, ethyl- and propyl parabens and their sodium salts (E214-219) are conditionally permitted for use in a limited number of foods in combination with either sorbates or sorbates and benzoates.

In 1974, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) established an ADI (acceptable daily intake for humans) of 0-10 mg/kg bw, as the sum of methyl, ethyl and propyl p-hydroxybenzoic acid and their sodium salts. The ADI was based on chronic toxicity studies from the 1950 – 60´s on methyl, ethyl and propyl parabens in rats showing a no-observed-effect-level (NOEL) for all three parabens of 2% in the diet, equivalent to 900-1200 mg/kg bw/day. The effect observed at the higher dose level of 8% in the diet was decreased weight gain accompanied by depression and death. JECFA was unable to establish an ADI for butyl paraben (JECFA, 1974).

The EC Scientific Committee for Food (SCF) evaluated the parabens in 1994 (SCF, 1996) and established a temporary ADI of 0-10 mg/kg bw, as the sum of methyl, ethyl and propyl p-hydroxybenzoic acid and their sodium salts. The temporary ADI was based on the same long-term studies in rats with methyl, ethyl and propyl paraben, which had been previously used by JECFA (JECFA 1974). The ADI was made temporary because the SCF considered that the toxicological information available showed some inadequacies and uncertainties.

Reproduction and teratogenicity studies were only available for ethyl paraben in the rat fed at levels up to 10% in the diet. No adverse effects on reproductive performance were reported but the findings with respect to fetal anomalies were equivocal with no clear dose-response relationship. In addition, the SCF noted that cell proliferation effects in the forestomach similar to those produced by BHA had been observed when certain alkyl esters of p-hydroxybenzoic acid were given in the diet in the form of a ground powder. The SCF therefore requested a new oral teratogenicity study in the rat using either free p-hydroxybenzoic acid or its methyl, ethyl or propyl ester and a cell proliferation study in the rat on the propyl ester of p-hydroxybenzoic acid given as a solution.

In 2000, the SCF reiterated its wish to review the safety of parabens (SCF 2000). However, at its last meeting in April 2003 the SCF noted that no data had been submitted by the food industry in support of the parabens and drew attention to its statement of October 2000, that the temporary ADI should be withdrawn if no further data are submitted (SCF 2003).

Because article 1(2) in the new modification of Directive 95/2 EC reads "Before 1 July 2004 the Commission and the EFSA shall review the conditions for the use of the additives E 214 to E 219" the Panel has been asked to provide an opinion on the safety of paraben usage in foods by 1 July 2004. Before undertaking this evaluation the Panel, in accordance with the SCF opinion of April 2003, sought confirmation that parabens were still being used in food. The Panel was informed that methyl, ethyl, and propyl parabens continued to be used in some sectors. The Panel therefore agreed that a re-evaluation would be necessary as parabens currently had a temporary ADI.

TERMS OF REFERENCE

Directive 2003/114/EC requires that the Commission and the European Food Safety Authority shall review the conditions for the use of additives E 214 to E 219 before 1 July 2004. The Scientific Panel on Food Additives, Flavourings, Processing Aids and materials in Contact with Food was asked to provide an opinion on the safety of paraben (E 214 to E 219) usage in foods.

ASSESSMENT

The SCF 1994 opinion on p-hydroxybenzoic acid alkyl esters and their sodium salts is attached as ANNEX 1.

Since the SCF evaluation in 1994, older studies on the developmental toxicity of methyl paraben, not considered by the SCF, have become available to the Panel. In addition, after a number of newer studies have shown that some parabens have oestrogenic activity *in vitro, in vivo* studies have been performed in order to elucidate the potential of parabens to interfere with normal reproductive function, particularly in males. Although butyl paraben is not used as a food additive, studies on this compound are included for comparison to the methyl, ethyl and propyl parabens.

No new studies on cell proliferation in the rat forestomach were available on the propyl ester of p-hydroxybenzoic acid given as a solution. The available studies are reevaluated and discussed.

Specifications

Commission Directive 96/77/EC of 2 December 1996 contains specific purity criteria on food additives other than colours and sweeteners including the parabens (E214-E219) (EU, 1996)**.**

Exposure

No recent estimates of the intake of parabens in Europe from their use as food additives were available.

Methyl-, ethyl-, and propyl parabens are permitted in the European Union as food additives in four categories of processed foods by Directive 95/2/EC: they are authorised for use *quantum satis* for the surface treatment of dried meat products and with a Maximum Permitted Level in jelly coatings of meat products such as pâté (1 g / kg), in confectionery - excluding chocolate (0.3 g / kg) and in liquid dietary food supplements (2 g /kg). Besides such uses of as food additives, parabens are widely used in cosmetics and toiletries. The EU regulation allows a content of up to 0.8% w/w (calculated as 4-hydroxybenzoic acid) for mixtures of esters e.g. methyl, ethyl, propyl and butyl paraben in cosmetic products. A preferential use of methyl → ethyl → propyl →butyl → benzyl paraben in various groups of cosmetic products was reported (Rastogi et al., 1995). These products may come into daily contact with the skin, hair and nails and lips, eyes, mouth and other mucous membranes. Parabens also have a long

history of use in a variety of pharmaceutical products intended for either injection, inhalation, oral, topical, rectal or vaginal administration (Soni et al., 2001, 2002).

Soni et al. (2002) assessed exposure to parabens from all sources in the USA. Total parabens exposure was estimated to be 77.5 mg/day (or 1.29 mg /kg /day for a 60 kg individual). Cosmetics and personal care products accounted for 50 mg (based on the assumption of daily use of 5 g of product containing 10 g / kg of parabens) and pharmaceutical products accounted for 25 mg (based on the assumption of daily ingestion of 5 g of drug containing 5 g / kg of parabens). Within this calculation, the estimate of the exposure related to food (2.5 mg) was a per capita availability based on production data in the USA and there is a large margin of uncertainty related to such estimates. Other far more conservative estimates of exposure through food were made available by the authors, based on the assumption that parabens are always present in the food categories where it may be used in the USA. Potential exposure estimates varied from 222 mg / day (Possible Average Daily Intake, based on average food consumption in the age class 2-65 years) to 466 mg /day (sum of 90th percentiles of potential intake based on three day consumption levels recorded for six food categories), i.e. less than 8 mg / kg bw in a 60 kg individual. These last estimates are likely to be conservative with respect to the European situation since parabens are allowed for use in a larger range of foods in the USA.

In Europe exposure via food may occur only through the consumption of four categories of food products (above mentioned). No refined exposure assessments were performed for parabens in the Report from the Commission on Dietary Food Additive Intake in the European Union (European Commission, 2001) since according to conservative estimates (tier 1) intake was under the temporary ADI of 10 mg /kg bw. However, these estimates probably did not take into consideration intake through meat products since Maximum Permitted Levels per kg of edible food are not specified for this category. The Panel noted that confirmation of the levels of parabens in meat products would be desirable.

Potential intake of parabens from meat products is difficult to assess. An estimate of exposure through dried meat products would require data on residue level in the edible portion of meat that received a surface treatment. In relation to jelly coating of meat products, no data are available to assess effective ingestion. A rough conservative estimate of

exposure through a single portion of pâté can be performed based on the hypothesis that 10% of the pâté portion is made with jelly coating and that the coating contains parabens at Maximum Permitted Level and is fully ingested. A 100 g portion of pâté would therefore lead to the intake of 10 mg of parabens i.e. 0.2 mg /kg body weight in a 60 kg subject.

Toxicological data

Absorption, distribution, metabolism, and elimination
No new data following peroral administration were available (see ANNEX 1)

Short-term studies of toxicity
No new data (see ANNEX 1)

Long-term studies of toxicity and carcinogenicity
No new data (see ANNEX 1)

Special studies on cell proliferation effects in the rat forestomach
Some subacute feeding studies have shown that parabens can induce cell proliferation in the forestomach of rats. In one of these studies, finely ground propyl p-hydroxybenzoate was given to groups of 5 weanling male Fischer 344 rats at 1 and 4% in the diet for 9 days. Propyl paraben induced proliferative changes in the squamous epithelium of the forestomach similar to, but less pronounced than the antioxidant butylated hydroxyanisole (BHA). Histologically and radioautographically, the degree of proliferation at 4% propyl paraben was similar to that observed at 0.5% BHA with a 2.5-fold increase in the thymidine labelling index compared to the controls. At 1% (equivalent to around 1 g/kg bw/day), the increase in the labelling index was only 1.5-fold and the only histological change was hyperplasia of the basal cells (Nera *et al*. 1984).

In another study on short term effects of various phenols and acids on the histological changes and the thymidine labelling index in the rat forestomach, finely ground substances of the p-hydroxybenzoic acid ester series were fed to groups of 5 weanling male Fischer 344 rats at 4% in the diet for 9 days. No proliferating activity was found with the free acid and the methyl ester. With the ethyl, propyl, and butyl ester, the activity in the prefundic region of

the forestomach increased with alkyl chain length, 4% butyl paraben in the diet being nearly as effective as 2% BHA (Rodrigues *et al*. 1986).

In contrast to these results, Shibata *et al*. (1990) found no changes in the forestomach and pyloric glandular epithelium, neither histopathologically nor in the bromodeoxyuridine labelling index following administration of 3% propyl paraben in the diet to older (6 week old) F344 rats for 8 weeks. Similarly, 3% propyl paraben in the diet of Syrian hamsters for 20 weeks did not produce histological changes in the forestomach (Ito and Hirose 1987).

Genotoxicity

Parabens have consistently been negative in bacterial mutagenicity test *in vitro*. It has been reported that methyl and ethyl paraben, but not propyl paraben, were able to produce chromosome aberrations *in vitro* in Chinese Hamster lung cells. However, methyl paraben did not produce chromosomal aberrations *in vitro* in human embryonic lung cells and methyl paraben did not induce aberrations in the chromosomes of bone marrow cells of mouse and rat, treated with high doses *in vivo* (Soni et al., 2002). (see ANNEX 1)

Developmental toxicity

Developmental toxicity studies with methyl paraben in mice, rats, hamsters and rabbits have now been made available (FDRL, 1972, 1973). Although they were performed some time ago, they were not available to the SCF at the time of its previous evaluations. Groups of 21-25 mice and rats were given methyl parabens orally by gavage at doses of 5.5, 25.5, 118 or 550 mg/kg bw/day on days 6-15 of gestation. Groups of 21-22 hamsters were given methyl parabens orally by gavage at doses of 3, 14, 65 or 300 mg/kg bw/day on days 6-10 of gestation. Groups of 12-20 rabbits were given methyl parabens orally by gavage at doses of 3, 14, 65 or 300 mg/kg bw/day on days 6-18 of gestation. In all 4 studies the vehicle used for methyl paraben was water and sham controls were given corn oil only by gavage. Dams were killed just before term and the contents of the uterus examined. All fetuses were examined for external abnormalities, then one-third of these for soft tissue abnormalities and two-thirds for skeletal abnormalities (mice, rats, hamsters), or all examined for both soft tissue and skeletal abnormalities (rabbits). There were no significant effects of treatment in any of the 4 studies on maternal body weight, pregnancy rate, number of implantations, live litter size, embryo or fetal mortality, fetal weight, external, soft tissue or skeletal abnormalities. Aspirin was used

as positive control in all 3 rodent studies and showed the expected reductions in fetal body weight (mice and rats) and increases in soft tissue and skeletal abnormalities (rats). 6-aminonicotinamide was used as positive control in the rabbit study and showed the expected reductions in fetal weight and increases in soft tissue and skeletal abnormalities. It should be noted that the top doses used in these studies could be considered somewhat low in that they were not reported to cause any maternal toxicity and had no effect on maternal body weights.

Special studies on oestrogenic effects of parabens

In vitro studies

Routledge *et al*. (1998) tested methyl, ethyl, propyl, butyl and 4-*n*-dodecyl paraben as well as p-hydroxybenzoic acid in the *in vitro* recombinant yeast oestrogen screen and found the butyl, > propyl, > ethyl and > methyl ester to be weakly positive, whereas 4-n-dodecyl paraben and 4-hydroxybenzoic acid were without activity. Methyl paraben was approximately 2,500,000-fold less potent than 17β-oestradiol. The magnitude of the oestrogenic response increased with alkyl group size and the ethyl, propyl and butyl esters had potencies of 150,000-fold, 30,000-fold and 10,000-fold less than 17β-oestradiol, respectively. The effect of propyl paraben and butyl paraben was inhibited by addition of the antioestrogen 4-hydroxy tamoxifen, demonstrating that these compounds had to interact with the oestrogen receptor in order to show oestrogenic activity. In a competitive binding assay it was also found that un-metabolised butyl paraben was able to compete with ^{3}H-oestradiol for binding to the rat oestrogen receptor with an affinity approximately five orders of magnitude lower than diethylstilboestrol and between one or two orders of magnitude less than 4-nonylphenol.

Using a standardised oestrogen receptor competitive binding assay Blair *et al*. (2000) tested 7 parabens for their ability to displace ^{3}H-oestradiol from the oestrogen receptor (obtained from uteri from ovariectomised Sprague-Dawley rats) and calculated their relative binding affinity (RBA). 2-Ethylhexyl paraben (RBA = 0.018%) was the most potent paraben tested followed by heptyl (0.008%), benzyl (0.003%), butyl (0.0009%), propyl (0.0006%), ethyl (0.0006%), and methyl paraben (0.0004%). For comparison RBAs for 4-nonylphenol and bisphenol A in this assay were 0.035% and 0.008%, respectively.

Okuba *et al*. (2001) found that the *in vitro* oestrogenic activity of parabens increased in the order methyl, ethyl, propyl, butyl, isopropyl and isobutyl parabens by assaying oestrogen receptor dependent proliferation of human MCF7 breast cancer cells. Their potencies were 10^5 to 10^7 times lower than that of 17β-oestradiol. Using a competitive binding assay it was also shown that the parabens had similar relative (to diethylstilboestrol) binding affinities (RBA) to the human oestrogen receptors α and β. The RBA values for the parabens ranged from 0.01% to 0.1% of that of diethylstilboestrol.

Byford *et al*. (2002) also reported on the oestrogenic effects of methyl, ethyl, propyl and butyl parabens in oestrogen-sensitive human MCF7 breast cancer cells. Competitive inhibition of ^{3}H-oestradiol binding to MCF7 cell oestrogen receptors could be detected at 1,000,000-fold molar excess of butyl paraben (86% inhibition), propyl paraben (77%), ethyl paraben (54%) and methyl paraben (21% inhibition). At concentrations of 10^{-6} M the parabens also increased the expression of oestrogen-regulated genes in the MCF7 cells and increased the proliferation of the cells in monolayer culture. Using a similar experimental set-up isobutyl paraben and benzyl paraben were also shown to be oestrogenic in the MCF7 cells (Darbre *et al*., 2002, 2003). Competitive inhibition of ^{3}H-oestradiol binding was detected at 100,000-fold molar excess of isobutyl paraben (81% inhibition) and benzyl paraben (57% inhibition). At concentrations of 10^{-5} M these parabens maximally increased the expression of oestrogen-regulated genes in the MCF7 cells and increased the proliferation of the MCF7 cells in monolayer culture.

Rajapakse *et al*. (2002) using *in vitro* assays have demonstrated that combinations of xenooestrogens, which included benzyl-paraben, may produce an oestrogenic effect even though the concentrations of each chemical were below their individual thresholds for effect in the assay.

In vivo studies

Routledge *et al*. (1998) also tested methyl and butyl parabens in uterotrophic assays using both immature and ovariectomised (OVX) rats following oral and subcutaneous administrations. OVX rats were only dosed subcutaneously. Methyl paraben at oral or subcutaneous doses up to 800 mg/kg bw/day for three days did not increase the uterus weight in immature rats and failed to increase uterus weight and vaginal cornification in OVX rats.

Butyl paraben did not produce a statistically significant increase in uterus weight in immature rats following oral administration of up to 1200 mg/kg bw/day for three days. However, subcutaneous administration of doses between 400 and 800 mg/kg bw/day significantly increased uterus wet weights in immature rats. In the OVX rats subcutaneous doses of 1000 – 1200 mg/kg bw/day were needed to produce statistically significant uterotropic effects.

Subcutaneous administration of 1.2 or 12 mg isobutyl paraben/mouse/day for 3 days (Darbre *et al*., 2002) and topical administration of three daily doses of 33 mg benzyl paraben/mouse (approximately 2000 mg/kg bw/day) to the dorsal skin (Darbre *et al*., 2003) increased the uterine weight in immature female CD1mice. Topical administration of three daily doses of 10 mg benzyl paraben/mouse (approximately 750 mg/kg bw/day) to the dorsal skin of immature mice did not significantly increase uterine weight.

Lemini *et al*. (1997) investigated the oestrogenic activity in CD1 mice of *p*-hydroxybenzoic acid which is the main metabolite of the *p*-hydroxybenzoic acid esters in mammals. They reported a dose-dependent response on vaginal cornification and uterotrophic activities in both immature and adult OVX mice seen after subcutaneous administration of 5 mg/kg bw/day for three consecutive days. The potency was calculated to be approximately 1000-fold less than that of 17β-oestradiol. However, this oestrogenic effect of *p*-hydroxybenzoic acid could not be confirmed by Hossaini *et al*. (2000). They examined the oestrogenic activity of methyl, ethyl, propyl and butyl parabens and their shared main metabolite *p*-hydroxybenzoic acid in a mouse uterotrophic assay. Immature B6D2F1 mice were treated with oral or subcutaneous doses of the test compounds for three consecutive days. *p*-Hydroxybenzoic acid and butyl paraben were also tested by the subcutaneous route in a rat uterotrophic assay. In the mouse assay, none of the compounds tested produced any oestrogenic response at oral and subcutaneous doses up to 100 mg/kg bw/day, and for ethyl paraben even at an oral dose of 1000 mg/kg bw/day. In immature Wistar rats, subcutaneous administration of 5 mg *p*-hydroxybenzoic acid/kg bw/day was inactive whereas butyl paraben produced a weak oestrogenic response at 600 mg/kg bw/day.

Special studies on effects of parabens on the male reproductive system

Kang *et al*. (2002) injected pregnant Sprague-Dawley rats subcutaneously with daily doses of 100 or 200 mg butyl paraben/kg bw from gestational day (GD) 6 to postnatal day (PND) 20 and the offspring were examined at PND 21, 49, 70 and 90. In the group exposed to 200 mg/kg bw/day, the proportion of pups born alive and of pups surviving to weaning were decreased. In both treatment groups, the body weights of female offspring were significantly decreased at PND 49, 70 and 90. The weights of testes, seminal vesicles and prostate glands and the days to vagenal opening were significantly decreased in male and female offspring, respectively on PND 49, but not on PND 70 or 90, in rats exposed to 100 mg/kg bw/day. No such effects were observed after 200 mg/kg bw/day. The weights of female reproductive organs were not affected. The sperm count and the sperm motile activity in the epididymis were decreased at PND 90 after 100 and 200 mg/kg bw/day. Testicular expression of oestrogen receptor mRNAs was increased in the 200 mg/kg bw/day group at PND 90.

In contrast to diethylstilboestrol, ethinyl oestradiol and tamoxifen, daily subcutaneous injection of 2 mg butyl paraben/kg bw to male neonatal Wistar rats on postnatal days 2 – 18, did not produce any changes in testis weight, distension of the rete testis and efferent ducts, epithelial cell height in the efferent ducts or immunoexpression of the water channel aquaporin-1. Animals were examined for these oestrogen sensitive parameters on days 18, 25, 35 and 75. Treatment with genistein, octylphenol or bisphenol A at high doses produced only very minor decreases in epithelial cell height in the efferent ducts (Fisher *et al*., 1999).

Oishi (2001, 2002a) examined the effects of dietary administration of butyl paraben to post-weaning male rats and mice. In the mouse study, butyl paraben was administered to groups of eight 4-week-old male Crj:CD-1 mice at doses of 0.00%, 0.01%, 0.10% and 1.00% in the diet for 10 weeks, corresponding to average butyl paraben intakes of 14.4, 146 and 1504 mg/kg bw/day, respectively. There were no effects on the weights of liver, ventral prostates, seminal vesicles, and preputial glands. However, the weights of the epididymides were significantly increased in the high-dose group. A dose-dependent decrease of both round and elongated spermatid counts was observed in the seminiferous tubules. The number of spermatogonia and spermatocytes were not different from the controls. Serum testosterone was significantly decreased at the highest dose (Oishi, 2002a). In the rat study butyl paraben was administered to groups of eight 3-week-old Wistar rats at doses of 0.00%, 0.01%, 0.10% and 1.00% in the diet for eight weeks, corresponding to average butyl paraben intakes of 10, 100 and 1000

mg/kg bw/day, respectively. The weights of the epididymides were significantly decreased in the mid- and high-dose groups. The cauda epididymal sperm reserve of all treated groups was decreased. The sperm count of the high dose group was 58.2% of the control value. The daily sperm production in the testis was also significantly lower in all treated groups. Serum testosterone was significantly decreased at the mid and high doses (Oishi, 2001). The author speculated that butyl paraben may exert a direct toxic action on the testes and reproductive tract because it has been reported to show potent spermatocidal activity on human spermatozoa *in vitro* due to impairment of the sperm membrane function. In this respect, butyl paraben was three and eight times more potent than propyl paraben and methyl paraben, respectively (Song *et al*., 1989, 1992).

In a similar experiment Oishi (2002b) administered propyl paraben to groups of eight 3-week-old male Wistar rats at doses of 0.00%, 0.01%, 0.10% and 1.00% in the diet for four weeks, corresponding to average propyl paraben intakes of 10, 100 and 1000 mg/kg bw/day, respectively. The basal diet used was a modified AIN93G diet devoid of soy-based phytoestrogens. There were no effects on the weights of the reproductive organs. The cauda epididymal sperm reserve and concentrations were decreased in the mid and high dose groups. The sperm count of the high dose group was approximately 50% of the control value. The daily sperm production in the testis was also significantly lower in all treated groups (about 70% of control), however no dose-response relationship was observed. Serum testosterone was significantly decreased in the high dose group.

Recently, Oishi (2004) also tested methyl and ethyl parabens for effects on secretion of sex hormones and the male reproductive function. Methyl or ethyl parabens were administered to groups of eight 3-week-old male Wistar rats at doses of 0.00%, 0.1%, and 1.0% each in the diet for eight weeks, corresponding to average intakes of 103 and 1030 mg methyl paraben/kg bw/day and 103 and 1043 mg ethyl paraben/kg bw/day, respectively. There were no effects of either compound on weights of the reproductive organs, on sperm counts in the testes and epididymides, and on the morphological examinations of spermatogonia, spermatocytes, round spermatids and elongated spermatids. In addition, serum concentrations of testosterone, LH and FSH were not affected.

Special studies on residues in human tissue

Recently, Darbre *et al.* (2004) reported the presence of small amounts of parabens in tissue samples from human breast tumours. Breast tumour samples were applied from 20 patients and analysed by HPLC and tandem mass spectrometry for methyl, ethyl, propyl, butyl, isobutyl, and benzyl paraben. The reported mean total paraben concentration was 20.6 ng/g tissue (blank value of 33.8 ng/g subtracted). Methyl paraben was present at the highest level (12.8 ng/g) followed by propyl paraben (2.6 ng/g). Benzyl paraben could not be detected. The authors calculated a corrected (for 50% recovery) average total paraben concentrations of 100 ng/g and compared this with the level of approximately 150 ng/ml (10^{-6} M) of propyl, butyl, and isopropyl paraben that have stimulated oestrogen-dependent growth in MCF7 human breast cancer cells. The authors argue that it is not inconceivable that the levels of parabens measured could exert oestrogenic effects on cells in the human breast. However, most of the parabens present were not these three parabens, but the much less potent oestrogenic methyl paraben. Instead the average level of these three more active parabens could be estimated at about 15 ng/g (corrected for 50% recovery), which is comparable to a concentration of 10^{-7} M, not found active in the MCF7 assay.

Discussion

In some studies, high concentrations of propyl paraben and butyl paraben in the diet have been shown to induce cell proliferation in the forestomach of rats. Ethyl paraben was less active and methyl paraben had no effect. Other studies with similar concentrations of propyl paraben in the diet of rats and hamsters did not show this effect.

The potential significance of proliferative changes in the forestomach of rats to man depends on whether or not the inducing substances are genotoxic. In contrast to other substances known to cause cell proliferation in the forestomach of rats, parabens are not genotoxic. This has been shown by several *in vitro* studies covering both point mutations and chromosome aberrations and by an host mediated and a dominant lethal assay *in vivo*.

The proliferative effect of parabens will only occur above a certain threshold. While a small increase of cell proliferation in the forestomach epithelium of weanling rats was observed after 1% propyl paraben in the diet it should be noted that two other rat studies failed to

demonstrate such an effect at higher doses (0.9 – 1.2 g propyl paraben/kg bw/day) and longer duration. Available data in the USA and rough estimates based on European use levels suggest that intakes from food will probably be several orders of magnitude below such doses. However no exposure estimates could be performed in relation to exposure through dried meat products in which parabens may be used *quantum satis* as a surface treatment. .

The newly available studies on the developmental toxicity of methyl paraben in rats, mice, hamsters, and rabbits did not show any evidence of developmental toxicity up to and including the highest doses tested of 300 (rabbits) or 550 mg/kg body weight/day (rodents).

Weak oestrogenic effects of parabens have been demonstrated in a number of *in vitro* systems. The studies have shown that parabens are able to bind to the oestrogen receptors α and β, to increase the expression of oestrogen-regulated genes and to stimulate proliferation of oestrogen-dependent mammalian cells. The oestrogenic potency increases with increasing length and branching of the alkyl side-chains in the following order: methyl < ethyl < propyl < butyl < isopropyl < isobutyl < benzyl < heptyl < 2-ethylhexyl paraben. However, p-hydroxybenzoic acid (without alkyl substitution) and n-dodecyl paraben (long side-chain) were inactive. Methyl paraben was approximately 2,500,000-fold less potent than 17β-oestradiol and the ethyl, propyl and butyl esters had potencies of 150,000-fold, 30,000-fold and 10,000-fold less than 17β-oestradiol, respectively.

The *in vivo* oestrogenic effects of parabens have been tested in uterotrophic assays employing either immature or ovariectomized mice and rats after oral, subcutaneous or dermal administration. Methyl paraben at doses up to 800 mg/kg bw/day for three days had no effect in rats and mice following oral or subcutaneous administration. Similarly, ethyl paraben and propyl paraben given orally or subcutaneously to mice at doses up to 100 mg/kg bw/day for three days were inactive. Oral administration of butyl paraben to rats and mice at doses up to 1200 mg/kg bw/day for three days had no uterotrophic effect, whereas subcutaneous administration of doses between 400 and 800 mg/kg bw/day significantly increased uterus wet weights in immature rats. In addition, subcutaneous administration of isobutyl paraben was found to produce an uterotrophic effect in the immature mouse.

Conflicting results have been reported for *p*-hydroxybenzoic acid, the common metabolite of all the parabens. One study reported an uterotrophic effect in mice after subcutaneous administration of 5 mg/kg bw/day for three days. However, this could not be confirmed in another study, where *p*-hydroxybenzoic acid at even higher dose levels was without effect in mice and rats. This latter result is in accordance with the lack of *in vitro* oestrogenic activity of *p*-hydroxybenzoic acid.

Butyl paraben, a paraben not used in food, reduced the numbers of sperm cells, impaired spermatogenesis, and reduced cell motility in the male offspring of rat dams that had been dosed subcutaneously with 100 and 200 mg butyl paraben/kg bw/day from gestation day 6 to postnatal day 20.

In experiments where butyl paraben was administered in the diet to juvenile rats and mice at doses of 10, 100 and 1000 mg/kg bw/day for up to eight weeks reduced numbers of sperm cells, impaired spermatogenesis, and reduced testosterone levels were reported. However, the weights of the epididymides increased in the mice at the high dose, but decreased in the rat at the mid- and high dose. Methyl, ethyl, and propyl parabens, the parabens used in food, have also been studied in juvenile rats for effects on sex hormones and male reproductive organs using comparable experimental designs.

Compared to butyl paraben, propyl paraben produced similar, but weaker, effects (reduced numbers of sperm cells, impaired spermatogenesis, and reduced testosterone levels) in juvenile rats after 100 and 1000 mg propyl paraben/kg bw/day after four weeks of dosing. In this case no effects were seen on the weights of the reproductive organs. However, reduced (about 70% of control) daily sperm production in the testis was also reported for the low dose group receiving 10 mg propyl paraben/kg bw/day, although no dose-response relationship was apparent for this effect. Since daily sperm production is part of a continuum of effects on the testis consistent with the testicular effects seen at the two higher doses these effects cannot be discounted. Therefore, the lowest dose, 10 mg propyl paraben/kg body weight/day, might be considered a LOAEL for propyl paraben.

In contrast to butyl and propyl paraben, methyl and ethyl paraben showed no effects on sex hormones and the male reproductive organs in juvenile rats at dose levels up to 1000 mg/kg body weight/day.

The finding of minute amounts of parabens in human breast tumour tissues has been linked by some to the development of breast cancer in humans. In particular the use of parabens in underarm antiperspirants and deodorants, rather than their use as food additives, have been implicated. However, these findings should be interpreted with great care. Firstly, it is not surprising that trace amounts of widely used compounds can be found in human tissues. In that respect, many persistent chemicals of potentially higher oestrogenic potency has been detected in human breast tissue, and in higher amounts, without any established link to cancer development. Secondly, based on problems with the analytical procedure (high background, low recovery) and the absence of appropriate controls, no conclusions based on the results are possible. Thirdly, no relationship between breast cancers in women aged 20-74 years and use of underarm antiperspirants and deodorants was found in a recent American case-control study, involving 813 cases and 793 controls (Mirick *et al*., 2002).

Conclusions and Recommendations

The SCF had previously requested an oral teratogenicity study in the rat. The Panel evaluated newly available studies on the developmental toxicity of methyl paraben in rats, mice, hamsters, and rabbits which were not available to the SCF when it made its request for a teratogenicity study. No evidence of developmental toxicity up to and including the highest tested doses of 300 or 550 mg/kg body weight/day were observed. The Panel concluded that no further data on developmental toxicity were needed.

The Panel also re-evaluated the proliferative effects of parabens on forestomach cells in rats, and concluded that the proliferative effect of parabens will only occur above a certain threshold and that the human exposure resulting from the use of parabens as preservatives in food will be several orders of magnitude below such doses.

Consequently the Panel considered that the study previously requested by the SCF on cell proliferation in the rat on the propyl ester of p-hydroxybenzoic acid given as a solution was no longer needed.

Several parabens have shown oestrogenic activity *in vitro*, with the methyl and ethyl parabens being much less potent than propyl paraben and in particular butyl paraben. However, no oestrogenic activity could be detected *in vivo* for methyl, ethyl, and propyl parabens in classical uterotrophic assays using oral or subcutaneous administrations of high doses to mice and rats. An *in vivo* uterotrophic effect was observed after subcutaneous injection of either butyl paraben or isobutyl paraben, which are not used as food additives. The common metabolite of parabens, p-hydroxybenzoic acid, was considered to be non-oestrogenic.

Dietary administration of propyl paraben to juvenile male rat for four weeks was reported to reduce the daily sperm production in the testis in all dose groups, including the lowest dose levels of 10 mg /kg body weight/day. At higher dose levels, reduced numbers of sperm cells, impaired spermatogenesis, and reduced testosterone levels were also observed. Thus, 10 mg/kg body weight/day was considered a LOAEL for propyl paraben. In contrast, methyl and ethyl paraben showed no effects on sex hormones and the male reproductive organs in juvenile rats at dose levels up to 1000 mg/kg body weight/day. Therefore 1000 mg/kg body weight/day was considered a NOAEL for both methyl paraben and ethyl paraben.

The Panel established a full group ADI of 0-10 mg/kg bw for the sum of methyl and ethyl p-hydroxybenzoic acid esters and their sodium salts on the basis of the NOAELs of 1000 mg/kg bw/day for each compound in long-term toxicity studies and studies on sex hormones and the male reproductive organs in juvenile rats. The Panel considered that propyl paraben should not be included in this group ADI because propyl paraben, contrary to methyl and ethyl paraben, had effects on sex hormones and the male reproductive organs in juvenile rats.
The Panel is unable to recommend an ADI for propyl paraben because of the lack of a clear NOAEL.
The Panel noted that human exposure resulting from the use of parabens in food in Europe has not been adequately assessed.

DOCUMENTATION PROVIDED TO EFSA

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Annex 1 SCF opinion from 1994.

The text below is from the Secretariat archives, the published version of the opinion can be found at http://europa.eu.int/comm/food/fs/sc/scf/reports/scf_reports_35.pdf

OPINION ON *P*-HYDROXYBENZOIC ACID ALKYL ESTERS AND THEIR SODIUM SALTS EXPRESSED ON 25 FEBRUARY 1994

1. Terms of reference

To advise on the safety in use of *p*-hydroxybenzoic acid alkyl esters and their sodium salts as food additives.

2. Introduction

p-Hydroxybenzoic acid alkyl esters and their sodium salts (parabens, PBs) have been extensively used as preservatives in food over many years. In 1974 the Joint FAO/WHO Expert Committee on Food Additives (JECFA) evaluated several parabens and established an ADI of 0-10 mg/kg bw, as the sum of ethyl, methyl and propyl *p*-hydroxybenzoic acid and their sodium salts. JECFA was unable to establish an ADI for the butyl ester of *p*-hydroxybenzoic acid. The Scientific Committee for Food (SCF) has not previously established an ADI for any of the parabens. However, the SCF did consider one of the parabens, sodium methyl *p*-hydroxybenzoate, in 1975 and confirmed its agreement with the JECFA evaluation. Accordingly, sodium methyl *p*-hydroxybenzoate was added to the EC list of permitted food preservatives which already included methyl *p*-hydroxybenzoic acid and ethyl *p*-hydroxybenzoic acid and its sodium salt.

3. Summary of metabolism and toxicity data

Many of the pharmacokinetic observations and toxicological studies on the parabens were carried out some years ago and would not fulfil present day criteria for conduct of studies. However, considering the parabens as whole, there is a considerable range of studies available and the Committee regards most of them as helpful for safety evaluation purposes. Absorption, metabolism and excretion has been studied in rats, rabbits, dogs and humans. The methyl, ethyl and propyl esters of *p*-hydroxybenzoic acid (Me-PB, Et-PB and Pr-PB) are well absorbed and the ester linkage is readily hydrolysed, as indicated by high plasma levels and early urinary excretion of free *p*-hydroxybenzoic acid, *p*-hydroxyhippuric acid and other metabolites such as ester glucuronides and ether sulphates. Urinary excretion of unchanged

esters of *p*-hydroxybenzoic acid is very low, usually less than 1% of the administered dose. Limited *in vitro* data on the butyl ester (Bu-PB) suggest it may follow a different metabolic pathway. Studies with prolonged dosing in dogs show no evidence of accumulation of either parent compounds or metabolites in the tissues.

Acute toxicity is only seen at high doses. All the parabens produce similar symptoms with rapid onset of ataxia, paralysis and central nervous system depression, resembling anaesthesia, suggesting their toxicity is related mainly to the free acid. With non-fatal doses recovery is prompt.

Subchronic toxicity studies on Me-PB, Et-PB and Bu-PB and chronic toxicity studies on Me-PB, Et-PB and Pr-PB have been conducted in rats. The no-effect level for all four parabens was 2% in the diet, equivalent to 0.9-1.2 g/kg bw/day. Effects occurring at a much higher dietary inclusion level of 8% were decreased weight gain (Me-PB and Pr-PB) accompanied by depression and death (Et-PB and Bu-PB). Doses intermediate to 2% and 8% were not tested. Me-PB and Pr-PB have also been tested at 500 and 1000 mg/kg bw/day given for approximately one year in the dog with a no-effect level of 1000 mg/kg bw/day for both esters. Bu-PB has been tested in the mouse at levels up to 10% in the diet for 6 weeks. The no-effect level in the mouse was 0.6% (equivalent to around 0.9 g/kg bw/day).

Several *in vitro* mutagenicity studies covering both point mutations and chromosome aberrations, and an *in vivo* host mediated assay and dominant lethal assay provided no evidence of genotoxicity of Me-PB. Pr-PB and Bu-PB were not mutagenic *in vitro*. No mutagenicity data are available for Et-PB.

The only long-term study specifically designed to address carcinogenicity was conducted on Bu-PB in mice, given up to 0.6% in the diet for two years. It reported no significant difference in tumour rates between treated and control animals but was inadequate for assessment due to early deaths in treated and control groups and relatively high incidence of some tumours in the control group.

Reproduction and teratogenicity studies in the rat using Et-PB at levels up to 10% in the diet found no adverse effects on reproductive performance but the findings with respect to fetal anomalies were equivocal, the reported anomalies showing no clear dose-response relationship. There are no other reproduction studies available for the parabens.

A number of special studies on cell proliferation in the forestomach and glandular stomach of rats have been carried out using finely ground powdered parabens, fed for 9 days at up to 4% in the diet. Me-PB was without activity, Et-PB showed minimal activity, whilst Pr-PB and

Bu-PB induced cell proliferation in the pre-fundic region of the forestomach. The potency depended on the alkyl chain length; 4% Pr-PB and Bu-PB had activities equivalent to 0.5% and 2% dietary BHA respectively.

4. Conclusions and recommendations

The data available give adequate reassurance that use of the methyl, ethyl and propyl esters of *p*-hydroxybenzoic acid and their sodium salts as food preservatives is temporarily acceptable. However, the toxicological information available shows some inadequacies and uncertainties and further studies along the following lines are needed:

– Since cell proliferation effects in the forestomach similar to those produced by BHA have been observed when certain alkyl esters of *p*-hydroxybenzoic acid were given in the diet in the form of a ground powder, a cell proliferation study in the rat on the propyl ester of *p*-hydroxybenzoic acid given as a solution should be carried out.

– In view of the equivocal findings in the existing oral teratogenicity study, a new oral teratogenicity study in the rat using either free *p*-hydroxybenzoic acid or its methyl, ethyl or propyl ester.