Scientific Committee on Food   **SCF/CS/ADD/CONS/50 Final**

Opinion of the Scientific Committee on Food

on

4-Hexylresorcinol

(expressed on 5 March 2003)

OPINION ON 4-HEXYLRESORCINOL

Terms of Reference

To advise the Commission on the safety in use of 4-hexylresorcinol to prevent melanosis of crustaceans.

Background

4-hexylresorcinol (4HR) was authorised in France on 13.11.1997 to prevent melanosis in crustaceans under Directive 89/107/EEC Art.5 on a national provisional basis. Its inclusion in the Community legislation is now requested in accordance with the conditions of Article 5. The substance was evaluated in 1994 by the French Commission de Technologie Alimentaire (2) as a replacement for the conventional sulphite treatment to prevent melanosis in crustaceans, mainly shrimps and lobsters. It offered certain technological advantages and the positive recommendation included a residue limit of 3 mg/kg in the raw product. Subsequently the Conseil Superieur d'Hygiène Publique de France recommended the use of 4HR as a substitute for sulphite, provided the material corresponded to the Joint FAO/WHO Expert Committee on Additives (JECFA) specification of 1995 and set a maximum residue limit of 2 mg/kg in the consumable portion of crustaceans (3). Thereafter, the French Ministry of Agriculture and Fisheries together with the French Ministry of Health issued a regulation on 4.11.1997 permitting the use of 4HR under specified conditions for prevention of melanosis of raw crustaceans with a maximum residue limit of 2 mg/kg for the two year-period allowable under Community legislation (5). The application was reviewed by the SCF in 1999 when further information on some aspects and an unscheduled DNA synthesis (UDS) study were requested. The new information required by the Committee has now been received. Together with the data previously supplied it constitutes the basis of this opinion.

4HR has a long history of human pharmaceutical use as a topical skin and mucosal disinfectant for treatment of superficial wounds, and as component of soaps, handwashes and skin cleaners. 4HR has been used in dilute solutions as antiseptic gargles or throat spray, and in lozenges. It was formerly used as anthelminthic for parasitic worm infestation in animals and in children less than 10 years of age at doses of 100 mg/year of age (9). In the mid-1920s it was used as urinary antiseptic (29,30). 4HR is still marketed today as an ingredient of certain throat lozenges.

Introduction

Black spots (melanosis) form in the shell of raw, refrigerated and frozen crustaceans within a few hours after harvesting by the action of polyphenol oxidase on naturally occurring colourless phenols resulting in coloured quinones. These subsequently polymerise to insoluble dark melanins. Refrigeration alone does not prevent but only slows this process as the enzyme remains active during refrigeration, ice storage and post-freeze thawing. Currently, a one minute dip into a 1.25% sulphite solution is used to inhibit melanosis. Sulphite, being a reducing agent, reacts chemically with the black spot precursors, whilst 4HR acts as a specific inhibitor of polyphenol oxidase. According to the petitioner, a solution containing 50 mg/L 4HR has been shown to prevent melanosis to the same degree as a 12.5 g/L sulphite solution.

Technical information

The JECFA specification for 4HR lays down a purity of 98% and includes an AOAC colorimetric method of analysis (4).   Similar specifications for 4HR appear in the pharmacopoeias of the USA, UK, Fl, NL and I.   The commercial product contains sodium chloride and tricalcium phosphate.   The thermal stability of the 4HR residues is unaffected by boiling for 5 minutes in tapwater, the usual treatment of fresh shrimps before consumption (31).

Shrimps are treated by dipping them for 1 minute into a tank containing an aqueous solution of 50 mg/L 4HR and this is not followed by post-dip rinsing.   4HR residues in the meat of shrimps range from <1 to 2 mg/kg   (31).   They decline rapidly during 5 days after treatment irrespective of the initial concentration of the dipping solution (32).   4HR at the concentrations proposed for use prevents browning of shrimp meat for about 4-5 days (32).

Microbiological consideration

Due to the low concentration of 4HR in the dipping solution (50 mg/L) used for preventing melanosis in shrimps no disinfectant effects are expected against any potential contaminating micro-organisms. For the latter purposes concentrations of about 0.5-1 % are required.

Exposure

The acute exposure resulting from the consumption of 150 g shrimps containing a maximum residue of 2 mg/kg crustacean meat is 0.3 mg (equivalent to approximately 5 µg/kg bw) which is about 1/100 that from the use of throat lozenges. Chronic intakes of 4HR, based on US consumption data for a 70 kg adult, range from 0.01- 0.03 µg/kg bw/day for shrimps having a residue ranging from 0.1- 0.9 mg/kg. The equivalent figures for the 90th percentile are 0.03-0.07 µg/kg bw/day (1).   Exposure from throat lozenges, assuming 4 mg/lozenge and a consumption of 6 lozenges/day would amount to 0.34 mg/kg bw/day (17).   The JECFA estimate of chronic intake, based on residue levels of 1 mg/kg crustacean meat, is 1-8 µg/day (equivalent to 0.01 - 0.11 µg/kg bw/day) (24).

Absorption, metabolism and excretion

This was studied in dogs and human volunteers, but kinetic data or plasma levels have not been investigated. Metabolites have not been studied in detail.

Dogs excrete between 10% - 30% in the urine and 70% - 80% in their faeces (6). About 1/3 of the oral dose was absorbed from the gut, urinary excretion of absorbed 4HR being rapid and almost complete within 6 hrs. Of the urinary metabolites 95% were identified as a conjugate of 4HR with sulphuric acid. Most of the unabsorbed 4HR-fraction appeared in the faeces as unchanged 4HR (7).

In an early study in humans, orally administered 4HR was said to be excreted as 18% in the urine and 64% in the faeces (7).

Toxicological data

Acute Toxicity

The oral LD_{50} of 4HR in the rat, rabbit, guinea pig and dog ranged from 140 to >5000 mg/kg bw (4,9,7,10,11,23). In cats, which are known to be more sensitive to phenolic compounds the oral LD_{50} ranged from >60 - <260 mg/kg bw (8).

Prominent acute toxic effects observed were congestion and necrosis of the gastrointestinal mucosa, occasional congestion of liver, heart and kidneys with focal necrosis and hyaline degeneration of renal tubular epithelium (8,9,10,11,25).

Adverse acute effects in humans exposed HR have been reported as irritation and erosion of the gastric and intestinal mucosa. Similar acute effects were seen after topical exposure of the respiratory mucosa and of the skin (25,26,27).

Using Caco-2 cells as model system for the intestinal epithelium no cytotoxicity was noted with concentrations up to 50 μg 4HR/ml medium. No significant inhibition of protein synthesis was seen up to 100 μg 4HR/ml medium (32).

Allergenicity/skin sensitisation

Tests in guinea pigs showed that 4HR was not sensitising when applied topically (19).

One case of contact dermatitis following occupational exposure has been reported but there was no cross-reaction with resorcinol (18). In a further study on cross-reactivity the administration of 4HR to 7 resorcinol-sensitive patients gave a positive result in 2 cases (20).

Subacute toxicity

In a 16 day-study in B6C3F1 mice, with oral doses ranging from 31.3 - 500 mg/kg bw/day no compound-related effects or body weight changes were seen (12).

In a 16 day-study in F344/N rats, with oral doses up to 500 mg/kg b.w./day only males showed a reduced body weight at 250 and 500 mg/kg bw/day No other toxicologically relevant compound-related findings were noted (12).

Subchronic toxicity

In a 13 week gavage study in B6C3F1 mice, using 4HR in corn oil at doses ranging from 62.5 - 1000 mg/kg bw/day all males and 9 out of 10 females given 1000 mg/kg bw/day died within 1 week. Only males showed reduced body weight at 250 and 500 mg/kg bw/day. A dose-related increase in mild to moderate nephropathy was the only other toxicologically relevant compound-related finding (12). A no-observed-effect-level (NOEL) for nephropathy could not be experimentally determined but the authors calculated a NOEL of 11 mg/kg bw/day by extrapolation from the dose-response curve, if the doses and results of the 2 week study were included (12).

In a 13 week gavage study in F344/N rats, using 4HR in corn oil at doses ranging from 63 - 1000 mg/kg bw/day all rats given 1000 mg/kg b.w./day died during the first week. Body weight was significantly reduced in males from doses of 125-500 mg/kg bw/day and in females from doses of 250-500 mg/kg bw/day. There was occasional diarrhoea, cachexia, nasal discharge, alopecia and ocular irritation (12). Males given 250, 500 and 1000 mg/kg/bw/day showed hypoplasia of seminal vesicles; hypospermatogenesis was observed at the lethal dose of 1000 mg/kg/bw/day only. There were no other treatment-related gross or histopathological findings (12). The NOEL for the effect on seminal vesicles in this study was 125 mg/kg bw/day. The possible influence of the observed weight loss on this finding is not clear (12).

Chronic toxicity/carcinogenicity

Mouse

Three groups of 50 male and 50 female B6C3F1 mice were given corn oil solutions of 4HR by gavage 5 days per week for 102 weeks at doses of 0, 63 and 125 mg/kg bw/day. Survival of test groups was comparable to controls but body weight gain was decreased for males and females of all test groups. No compound-related clinical effects were noted. The only non-tumorigenic lesions noted were osteosclerosis and nephropathy in both sexes. Osteosclerosis was manifest as focal or multifocal excess of cancellous bone with immature connective tissue and few haematopoietic cells. The incidence was increased in high dose males and females (12,16).

Nephropathy was manifest as mild focal tubular atrophy to severe atrophy with tubular cysts in the renal cortex, tubular regeneration, dilated tubular lumen and Bowman's space, and inflammatory infiltration. In males the incidence of these renal lesions was higher in controls than in test animals. In females there was a clear dose-related increase in the renal lesions and it was of more severe degree in test animals than in controls and occurred in all test groups (12,16). The NOEL for osteosclerosis and nephropathy in male mice was 63 mg/kg bw/day. The NOEL for osteosclerosis in females was 63 mg/kg bw/day. A NOEL for nephropathy in females could not be established experimentally. The authors however estimated a NOEL of 11 mg/kg bw/day from a combined dose-response curve covering the subchronic and chronic studies, and by assuming that nephropathy in controls was unrelated pathologically to that induced by 4HR and not additive, and that the 4HR pathology was directly dose-related (12,16).

No tumorigenic effect was seen in females. However in males a statistically not significant increase in the incidence of phaeochromocytomas and focal adrenal medullary hyperplasia was noted (12,16). Only 1 malignant phaeochromocytoma occurred in a low dose mouse. The bilateral proliferative lesions occurred mostly in low dose males (12,16).

In males the incidence of Harderian gland tumours was 13.5% in the low dose group and 10% in the high dose group compared with 0% in controls. Re-evaluation of the slides by an independent pathologist acting on behalf of the sponsors suggested that all tumours were adenomas and that the incidence was similar to that of historical controls (3.7-10%) (12,16). The incidence of hepatocellular adenomas and carcinomas in male mice showed a negative trend, being lower in the test groups than in controls. The incidence of haemangiosarcomas and haemangiomas in both sexes of the top dose group was lower than in controls and the incidence of alveolar/bronchiolar adenocarcinomas and adenomas in females of the lower test dose group was less than that in controls (12).

In another study in BALB/c mice 0.1 ml of a 1% suspension of 4HR in gum tragacanth was applied intravaginally biweekly for 31 weeks. One treated mouse developed a squamous carcinoma of the vagina after 15 months but none occurred in the gum tragacanth control group. Most treated mice had chronic inflammation of the genital mucosa (22).

Rat

Three groups of 50 males and 50 female F344/N rats were given a corn oil solution of 4HR by gavage 5 days/week for 103 weeks at doses of 0, 63, 125 mg/kg bw/day. No compound-related clinical signs and no significant differences in survival between the test and control groups were noted. Body weight was reduced by 7-11% only in males of the 125 mg/kg bw/day dose group, the body weights of all other test groups were similar to controls. There was a marginal increase in adenomas and adenocarcinomas of the anterior pituitary in females that was not considered to be of biological significance by the authors because of the variable incidence of these tumours in female F344/N rats (12). Three neural tumours occurred in high dose males, one in low dose males and one in controls. The incidence of mononuclear cell leukaemia in males and females showed a negative trend. Similar negative trends were noted with thyroid C-cell neoplasms in males, pancreatic islet cell tumours, mammary gland fibromas and endometrial stromal polyps. No significant trend was seen in non-neoplastic lesions in either sex (12).

Human data

A retrospective epidemiological study in women using daily 4HR mouthwashes did not show any association with oral cancer (28).

Genotoxicity

4HR was tested in two tests for gene mutation using *Salmonella typhimurium*. No evidence of mutagenicity was found (13,14). 4HR was tested in a gene mutation assay in *E.coli* W3110 pol A$^+$/p3478 polA$^-$. The assay was positive in disc diffusion and liquid suspension tests but negative in the microsuspension test (15). 4HR was tested for mutagenic activity in a mouse lymphoma L 5178Y assay. It was not mutagenic in the absence of metabolic activation (S9) but was mutagenic in the presence of S9 (12).

In a sister chromatid exchange assay using Chinese hamster ovary (CHO) cells 4HR gave a positive result in the absence of S9 but a negative result in the presence of S9 (12). 4HR was also tested for chromosomal aberrations in CHO cells. No increase in chromosomal aberrations was noted (12).

In an UDS assay in male rats doses of either 600 mg/kg bw or 2000 mg/kg bw in corn oil as vehicle were administered by gavage and hepatocytes isolated from the livers of treated animals after 2 hours or 14 hours exposure. No significant increases in UDS were noted. This assay provided evidence of the absence of *in vivo* genotoxic activity (34).

Reproductive and developmental toxicity

No studies on these aspects are available.

Although the chemical structure of 4HR raises an alert for possible endocrine disruptor activity, it should be recalled that only branched chain octyl and nonyl phenols have so far shown activity after oral administration, whereas 4HR is a shorter straight chain, hexyl derivative. A literature review has produced no evidence indicating endocrine disruptor activity of 4HR.

The effects on spermatogenesis and seminal vesicle size, noted in the subchronic rat studies are most likely due to indirect toxicity, as the doses required were accompanied by weight loss and/or death. 4HR has been used as a spermicidal agent in contraceptive creams. This activity was confirmed in an assay with human spermatozoa using cytoplasmic stripping of the spermatozoa as endpoint (21).

The use of 4HR in human therapeutic drugs has prompted studies looking for any association with congenital malformations. A literature review of reports on the use of anthelminthics as a group or of specific anthelminthic drugs including hexylresorcinols, and the induction of birth defects in humans failed to produce any reports of an association with hexylresorcinols (35). An epidemiological study on malformations in relation to antiparasitic agents, including hexylresorcinols, involving 3248 children with any malformation in 50282 mother-child pairs exposed during the first 4 months of pregnancy produced a hospital standardized relative risk for systemic exposure to hexylresorcinols of 0.70. For 2277 children with malformations showing uniform rates by hospital of the same 50282 mother-child pairs exposed during the first 4 months of pregnancy to the same systemic antiparasitic agents, including hexylresorcinols, the estimated hospital standardized relative risk for systemic exposure to hexylresorcinols was 1.05 (36).

Comments

Limited studies in dogs indicate that about 30% of an oral dose of 4HR is absorbed and that it is rapidly excreted in the urine. 4HR has a moderate acute toxicity due to its irritant properties. In subchronic and chronic studies in mice 4HR showed mild to severe nephrotoxicity over a dose range of 63 -1000 mg/kg b.w. with a NOEL of 11 mg/kg b.w. calculated by extrapolation from the available dose-response data. This calculated NOEL is several orders of magnitude above the estimated acute intake of 5 µg/kg bw/day from the proposed food use assuming maximum residue levels are present in the shrimp. No such effects were seen in the subchronic and chronic studies in rats.

The Committee noted that the in vitro genotoxicity data showed some inconsistencies but concluded from the negative UDS study that 4HR had no in vivo genotoxicity. As regards carcinogenicity the data in male mice were initially interpreted by the US National Toxicology Program (NTP) as equivocal evidence for a carcinogenic potential of 4HR. Re-evaluation of the histological material did not support the original interpretation of equivocal carcinogenic potential. The chronic studies in rats provided no evidence for a carcinogenic potential in this species. The Committee considered that 4HR was not carcinogenic.

Reproduction and developmental toxicity have not been investigated. Such data are not considered essential in this case given the single proposed use and the low exposure level. The subchronic studies in rats showed some evidence of an inhibitory effect on

spermatogenesis only at lethal doses several magnitudes higher than the likely exposure from consumption of 4HR residues in crustaceans. A literature review of reports on systemic exposure to anthelminthics and a large epidemiological study on systemic exposure to antiparasitic agents including hexylresorcinols during early pregnancy produced no evidence for any association with malformations.

Human studies have pointed to the possibility of contact dermatitis in persons who are allergic to resorcinol.

Conclusion

The available data do not allow the establishment of an ADI. Nevertheless the Committee considers 4- hexylresorcinol as toxicologically acceptable for the prevention of melanosis in shrimps under the conditions described provided residues in crustacean meat do not exceed 2mg/kg. For more extensive use or higher levels of application, further toxicological data would be required.

References

1. Coultor Food Science (1998). Dossier submitted to the EU Commission.

2. Commission de Technologie Alimentaire (1994). Record of 28th Session 7/7/1994.

3. Conseil Superieur d'Hygiène Publique de France (1996). Avis du 9/4/1996.

4. Joint FAO/WHO Expert Committee on Food Additives (1998). Specification for 4-hexylresorcinol, 51st session. FAO/FNP/52 Add 6, FAO. Rome.

5. French Ministries (1997). Regulation No.ECOC 9700166A.

6. Robbins, B.H. (1931). Quantitative studies on the absorption and excretion of hexylresorcinol and heptylresorcinol under different conditions. J.Pharmacol.Exper.Ther. 43, 325-333.

7. Robbins, B.H. (1934). Quantitative studies on the absorption and excretion of certain resorcinols and cresols in dogs and man. J.Pharmacol.Exper.Ther.,52,54-60.

8. Maplestone, P.A., Chopra, R.N. (1934). The effect of 4-hexylresorcinol on cats. Indian J.Med.Res.,21,519-521.

9. Lamson, P.D., Brown, H.W., Ward, C.B. (1935). Anthelminthic studies on alkyl-hydroxy-benzenes. I. Alkylpolyhydroxybenzenes. J.Pharmacol.Exper.Ther.,53,198-217.

10. Anderson, H.H., David, N.A., Leake, C.D. (1931). Oral toxicity of certain alkyl resorcinols in guinea pigs and rabbits. Proc.Soc.Exp.Biol.Med.,28,609-612.

11. Christensen, B.V., Lynch, H.J. (1933). The effect of anthelminthics on the host. I. Tetrachloroethylene. II. Hexylresorcinol. J.Pharm.Exper.Ther. 48, 311-316.

12. NTP (1988). Toxicology and carcinogenesis studies of 4-hexylresorcinol (CAS No.136-77-6) in F344/N rats and B6C3F1 mice (gavage studies). Technical Report Series No. 330. NIH publication No. 88-2586.

13 Mortelmans, K., Haworth, S.,Lawlor, T., Speck, W., Tainer, B., Zeiger, E.(1986). S*almonella* mutagenicity tests. II. Results from the testing of 270 chemicals. Environ.Muta. 8, (suppl.7), 1-119.

14 Cortinas de Nava, C.J., Espinosa, L.G., Zapata, M.A., Martinez, E., (1983). Mutagenicity of antiamebic and anthelminthic drugs in the *Salmonella typhimurium* microsomal test system. Mutation Res., 117, 79-91.

15 Espinosa-Aguirre, J.J., Aroumior, C., Meza, M.T., Cienfuegos, E., Cortinas de Nava, C., (1987). Genotoxicity of amebicide and anthelminthic drugs in *Escherichia coli* polA$^+$/polA$^-$. Mutation Res.,188, 111-120.

16. Environ (1990) Review and evaluation of carcinogenesis studies of 4-hexylresorcinol conducted by National Toxicology Program., prepared 15.12.1990

17. Frankos,V.H.,Schmit,DF.,Haws,L.C,McEvily,A.J,Iyengar,R,Miller,SA,

    Munro, I.C., Clydesdale, F.M.., Forbes,A.L., Sauer,R.,.(1991).
    Generally Recognised As Safe evaluation of 4-hexylresorcinol for use as a processing aid for prevention of melanosis in shrimp. Reg.Tox.Pharm., 14, 202.

18. Burrows, D., Irvine, J., (1982). Contact dermatitis to hexylresorcinol. Contact Dermatitis, 8, 71.

19. Baer, H., Watkins, R.C., Bowser, R.T., (1966). Delayed contact sensitivity to catechols and resorcinols. The relationship of structure and immunization procedure to sensitizing capacity. Immunochem., 3, 479-485.

20. Keil, H., (1962). Group reactions in contact dermatitis due to resorcinol. Archs. Derm., 86, 212-216.

21. Brotherton, J., (1977). Assessment of spermicides by a stripping technique against human spermatozoa. J. Reprod. Fert., 51, 383-391.

22. Boyland, E., Roe, F.J.C., Mitchley, B.C.V., (1966). Tests of certain constituents of spermicides for carcinogenicity in the genital tract of female mice. Br. J.Cancer, 20, 184-189.

23. Dittmer, D.ed.,(1959). Handbook of Toxicology. NAS-NRC, Washington, D.C.,5, 148-149.

24. Joint FAO/WHO Expert Committee on Food Additives (1996) WHO Food Additives Series No 35, WHO, Geneva.

25   Gosselin, R.E., Smith, R.P., Hodge, H.C.,eds.,(1984). Clinical Toxicology of Commercial Products. Williams & Wilkins, Baltimore/London, 5th ed. p.11-90.

26.   Gilman, A.G., Goodman, L.S., Rall, T.W., Murad, F., (1985). Pharmacological Basis of Therapeutics. Antiseptics and disinfectants. Chap.41, 7th ed., Macmillan Publishing Co., NY, NY, p.970.

27.   Hoover, J.E. ed. (1975) Remington's Pharmaceutical Sciences. 15th ed., Mack Publishing Co., Easton, PA. p. 1174.

28.   Wynder, E.L., Kabat, G., Rosenberg, S., Levenstein, M.., (1983).Oral cancer and mouthwash use. J. Natl. Cancer Insti., 70, 255-260.

29.   Leonard, V., (1924). Hexylresorcinol. The development and clinical application of a synthetic compound possessing the experimental requirements of an ideal internal urinary antiseptic. J.Urol. 12, 585-610.

30.   Leonard, V., Wood, A., (1925). The present status of hexylresorcinol as an internal urinary disinfectant. JAMA   85, 1855-1861.

31.   Iyengar, R.., Bohmont, C., McEvily, A.J., (1991). Hexylresorcinol Prevention of Shrimp Blackspot: Residue data. J.Food Comp.Anal., 4, 148.

32.   Guandalini, E., Ioppolo, A., Mantovani, A., Stacchini, P., Giovanni, C., (1998). 4- hexylresorcinol as inhibitor of shrimp melanosis: efficacy and residue studies; evaluation of possible toxic effect in a human intestinal in vitro model (Caco-2); preliminary safety assessment. Fd.Add.Contam., 15(2), 171-180.

33.   Slattery, S.L., Williams, D.J., Cusack, A., (1995). AQ sulphite-free treatment inhibits blackspot formation in prawns. Food Australia, 47(11), 509-514.

34.   Huntingdon Life Sciences (2001). 4-Hexylresorcinol rat liver DNA repair (UDS) test. Report No:ALS 217/013265, dated 27.July 2001. submitted to the EU Commission by Opta Food Ingredients Inc.

35.   Schardein, J.L. (2000) Chemically induced birth defects. 3rd Edition. Marcel Dekker, New York, USA.

36.   Heinonen, O.P., Slone,D., Shapiro, S., (1977). Birth Defects and Drugs in Pregnancy. Publishing Sciences Group, Littleton, Mass. USA.