Scientific Committee on Food

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25 July 2002

Opinion

of the Scientific Committee on Food

on quassin

(expressed on 2 July 2002)

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Terms of reference

The Committee is asked to advise the Commission on substances used as flavouring substances or present in flavourings or present in other food ingredients with flavouring properties for which existing toxicological data indicate that restrictions of use or presence might be necessary to ensure safety for human health.

In particular the Committee is asked to advise the Commission on the implication for human health of quassin in the diet.

Introduction

Previous evaluations

Quassin was classified as an active principle by the Committee of Experts on Flavouring Substances of the Council of Europe (CEFS) in 1981 with limits of 5 mg/kg in beverages and food, exceptions alcoholic beverages 50 mg/kg and lozenges 10 mg/kg (Council of Europe, 1981). In 1991 CEFS proposed to remove quassin from the list of active principles as "there is little evidence of quassin toxicity although most of the studies available are of poor quality" (Council of Europe, 1991a, 1991b). In 2002 CEFS decided to re-evaluate quassin for possible inclusion as an active principle as new toxicological data had become available (Council of Europe, 2002).

Current regulatory status

Quassin is listed in the 88/388/EEC Directive on Flavourings in Annex II with maximum limits of 5 mg/kg in foodstuffs and beverages, with exceptions 10 mg/kg in confectionery in pastille form and 50 mg/kg in alcoholic beverages (EEC, 1988).
In the U.S.A. Quassia (from *Picrasma excelsa* (sw.) Planch or *Quassia amara*, L.) is listed as "may be safely used in food" (CFR, 2002).

Chemical characterisation

Quassin is a diterpene lactone
Name: Quassin
Synonyms: (+)-Quassin; Nigakilactone D;
CAS no.:   76-78-8
EINECS no.: 200-985-9

Extracts of *Quassia amara* L. or *Picrasma excelsa* (Sw.) Planch may be referred to as
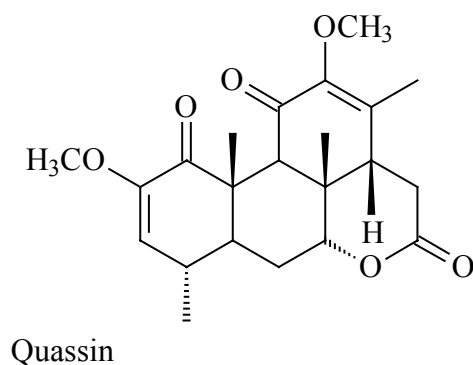   "quassin".
Name: Quassia extract
Synonym: "Quassin". Bitter wood extract.
CAS no.:   68915-32-2
EINECS no.: 272-809-9

Structure:

Quassin

Exposure assessment

There are no data available on use, use levels or exposure in Europe. It should be noted that extracts of *Quassia* are often simply referred to as "quassin" and may appear commercially under this name. "Quassia" is the dried stem wood of *Quassia amara* L. or of *Picrasma excelsa* (Sw) Planch (family Simarubaceae). Commercial "quassin" from *Quassia amara* is known to contain a mixture of the bitter principles (quassinoids), like quassin, neoquassin, and 18-hydroxyquassin (Robins *et al.*, 1984) whilst *P. excelsa* contains isoquassin, also known as picrasmin, instead of quassin as the major quassinoid (Leung and Foster, 1996).

Quassin can be used in food because of its very bitter properties (50 times more bitter than quinine) (Council of Europe, 1991a).
In the USA, *Quassia* extract may be used in beverages (3.4 mg/kg), alcoholic beverages (3.4 mg/kg) and in baked goods (50 mg/kg) (Hall and Oser, 1965).

Hazard identification / characterization

Absorption, distribution, metabolism and excretion

No data available.

Acute toxicity

No sign of acute toxicity was observed at any doses given orally to albino rats and mice up to 1000 mg/kg of aqueous *Quassia* extract. The quassin content was not given (Garcia *et al.*, 1997).

Sub-acute/sub-chronic toxicity

Two groups of rats aged approximately 2 years old (no strain or sex given), 10 rats in each group, were given dried *Quassia* extract (quassin content unknown) dissolved in water or water alone by gavage in doses of 50 mg/kg/day 6 days/week for 8 weeks.
All the rats were killed and the heart, liver, kidneys, adrenals and spleen were weighed. Body weights for the test and control animals were similar and remained roughly constant through out the study. Haematology tests revealed very little difference between the two groups. There were no significant differences in organ weights between groups either. However 2 of the control and one of the test rats died during the second week of the study, which the authors claim, was probably as a result of the inflammatory process of the bronchi which was found at post mortem. This could have been a result of the gavage method of dosing. (Margaria, 1963).

Chronic toxicity/Carcinogenicity

No data available

Genotoxicity

No data available

Reproductive and developmental toxicity

Pregnant rats were given dried *Quassia* extract at 100 mg/kg/day (presumably the same extract and by same method of dosage as in the subchronic study by Margaria, 1963) during the second half of pregnancy and through out the lactation period. The dosing period began at different times for each of the three rats in the test group i.e. at day 7, day 6 and day 5 before littering. No dosing details are given for the controls. The numbers of pups in each litter were as follows: 14, 8 and 12 in the three control animals and 10, 6 and 10 in the animals dosed on days 7, 6 and 5 before littering respectively. It would appear that there is a slight reduction in

the number of pups in the dosed groups but the significance of this is unclear. The weights of the pups in each litter were recorded at days 1, 7, 14 and 21 after birth. There were no significant differences in weights between test and control litters. The authors also say that there were no qualitative or quantitative differences between the test and control animals but do not provide any evidence to qualify this statement (Margaria, 1963).

Njar *et al.* (1995) have studied the effect of *Quassia amara* L. on the steroidogenesis in rat Leydig cells in an *in vitro* system. The crude methanol extract of the stem wood of *Quassia amara* L. at graded doses (50 – 250 µg/ml) inhibited both the basal and luteinising hormone (LH) stimulated testosterone secretion from rat Leydig cells in a concentration-dependent fashion. The composition, including content of quassin in the extract, is not given. Quassin and the alkaloid 2-methoxycanthin-6-one were isolated from the *Quassia* extract according to the method described by Njar *et al.* (1993). The two isolated compounds were studied in the same way as described above for the extract, and quassin proved to be the bioactive agent. Again, both the basal and the LH-stimulated testosterone production by the Leydig cells were inhibited in a dose related manner with doses from 5 ng/ml and up to 25 ng/ml of the isolated quassin. The inhibition of testosterone production was shown not to be caused by cytotoxic effects of the *Quassia* extract or of the isolated quassin.

In continuation of the above study, Raji and Bolarinwa (1997) have studied antifertility activity of *Quassia amara* L. in male rats. *Quassia amara* L. stem wood crude methanol extract and two compounds isolated from the extract, quassin and the alkaloid 2-methoxycanthin-6-one, were studied. The *Quassia* extract was given with the drinking water to male Wistar strain albino rats (200-220 g), five per group at doses corresponding to 100, 1000, and 2000 mg per kg body weight for eight weeks, then killed and examined as described below. A second set of groups of rats, also five per group, similarly treated as above, were subject to a recovery period of eight weeks without further treatment.

The composition, including the concentration of quassin and 2-methoxycanthin-6-one of the extract is not given. The isolated quassin and 2-methoxycanthin-6-one were applied in the same way as the *Quassia* extract, at doses corresponding to 0.1, 1.0 and 2.0 mg per kg body weight. The purity of the two compounds was not given. The control rats (five per group) received phosphate buffer saline. All rats had free access to food and water.

After the eight weeks of treatment the final body weight of the dosed groups did not differ significantly from the control groups, not either after the further eight weeks recovery period. The study demonstrated that the crude methanol extract of the stem wood of *Quassia amara* L. significantly reduced the weight of the testis, epididymis and seminal vesicle and significantly increased that of the anterior pituitary gland. Epididymal sperm counts, serum levels of testosterone, luteinising hormone (LH) and follicle stimulating hormone (FSH) were significantly reduced when the rats were treated with the extract. All these changes seemed to be restored completely eight weeks after withdrawal from the eight weeks of treatments.

Furthermore, the basal and LH-stimulated testosterone secretion from Leydig cells isolated from rats pre-treated with the extract was inhibited.

The viability of the Leydig cells was unchanged after the treatment (no lethal effect on the cells of the treatment). All the effects were shown at all three dose levels and no dose-relationship was demonstrated.

Quassin produced qualitatively and quantitatively biological actions similar to the *Quassia* extract while the effects of 2-methoxycanthin-6-one did not seem to differ from those of the control and the authors concluded that quassin appears to be the antifertility principle of *Quassia amara* L.

In the study is also briefly described the average litter size following mating with fertile female rats. It was 8.1 +/-1.1 in each of the groups, except in the groups treated with either crude extract of *Quassia* or quassin where litter sizes were zero and 3 +/-1, respectively. The number of animals per group was five. No more information was given, e.g. specification of the litter sizes in the different groups studied and whether mating also was performed after the eight weeks of recovery after treatment.

Human data

No data available

Other studies

From the results of experimental studies on quassinoids it has been suggested that several of these compounds might have anticarcinogenic, anti-malarial, antiviral or amoebicidal potential (Ajaiyeoba *et al*., 1999; Alvarez *et al*., 1995; Gillin *et al*., 1982; Lee*.,* 1999; Pierré *et al*., 1980; Trager and Polonsky, 1981). Generally, quassin is less active in these tests with quassinoids. Several quassinoids, including quassin, are shown to have antifeedant and insecticidal properties (Daido *et al*., 1993; Leskinen *et al*., 1984; Park *et al.,* 1987).

Summary of hazard identification / characterization

There are few studies on quassin toxicity and most of the studies available are of poor quality. There are neither chronic toxicity, carcinogenicity, genotoxicity or metabolism studies nor data on the levels of quassin present in the *Quassia* products used in the subacute feeding studies.

According to more recent studies by Njar *et al.* (1995) and by Raji and Bolarinwa (1997), *Quassia* extracts and quassin are shown to have antifertility activity both *in vivo*. In these studies effects were noted at a dose of quassin equivalent to 0.1mg/kg body weight (LOEL),

the lowest dose tested, and no NOEL could be established.. However the number of animals per group was only five in the rat study, no histopathology was performed and the information on the reproduction experiment part, including litter sizes, was insufficient. Quassin was shown also to inhibit steroidogenesis in rat Leydig cells in vitro and ex vivo in a concentration/dose related manner.

The extensive *in vivo* study by Raji and Bolarinwa (1997) had a number of unusual features:
- The significant effect on all antifertility parameters measured at all dose levels compared to the controls.
- The lack of dose-dependency.
- The high antifertility potency of quassin even at lowest dose (0.1 mg per kg body weight).
- The significant effect of *Quassia* extract and quassin on litter size compared to the control group.
- The recovery of all those parameters that were measured eight weeks after withdrawal of the treatment.

Risk characterisation

As data on exposure are missing and the toxicity data available are inadequate for establishing a NOAEL, a proper risk assessment can not be performed. However, two studies on reproductive toxicity give rise to toxicological concern.

To further investigate the antifertility potential of quassin and *Quassia* extract and to establish a NOEL for this effect a reproductive toxicity study in rats according to current guidelines is required. It should especially include male fertility parameters and include lower doses of quassin than in the former *in vivo* antifertility study.

In order to complete the risk assessment the Committee also requires further toxicological studies, at least studies to establish a NOEL in 90 day studies together with further studies on genotoxicity at the gene and chromosomal level in line with the general Guideline for Food Additives (SCF, 2001).

Usage and consumption data for relevant *Quassia* products, including the quassin content, should also be provided in order to make an exposure estimate of quassin.

The Committee noted that the potential intake from bitter alcoholic beverages only could lead to exposures similar to those reported to give rise to effects in experimental animals. Intakes from other foods and beverages could increase this intake still further.

References

Ajaiyeoba, E.O., Abalugo, U.I., Krebs, H.C., Oduola, A.M.J. (1999). In vivo antimalarial activities of *Quassia amara* and *Quassia undulata* plant extracts in mice. *J. Ethnopharmacology,* **67,** 321-325.

Alvarez, M., Paull, K., Monks, A., Hose, C., Lee, J.S., Weinstein, J., Grever, M., Bates, S., Fojo, T. (1995). Generation of a drug resistance profile by quantitation of *mdr*-1/P-glycoprotein in the cell lines of the National Cancer Institute Anticancer Drug Screen. *J. Clin. Invest.*, **95**, 2205-2214.

CFR 2002. US Code of Federal Regulations. Title 21. § 172.510.

Council of Europe (1981). Flavouring Substances and Natural Sources of Flavourings, 3rd Edition. Maisonneuve, Strasbourg, p.95.

Council of Europe (1991a). Committee of Experts on Flavouring Substances. 28th Session. Datasheet on quassin.

Council of Europe (1991b). Committee of Experts on Flavouring Substances. 29th Session. Record.

Council of Europe (2002). Committee of Experts on Flavouring Substances. 50th Session. Record.

Council of the European Communities (1988). Council Directive 88/388/EEC of 21 June 1988 on the approximation of the laws of the Member States relating to flavourings for use in foodstuffs and to source materials for their production, Official Journal of the European Communities, 15.7.1988, L184/61-67.

Daido, M., Fukamiya, N., Okano, M., Tagahara, K., Hatakoshi, M., Yamazaki, H. (1995). Antifeedant and insecticidal activity of quassinoids against diamondback moth (*Plutella xylostella*) *Biosci. Biotech. Biochem.*, **57**, 244-246.

Garcia, G.M., Gonzalez, S.M.C., Pazos, L.S. (1997). [Pharmacologic activity of the aqueous wood extract from *Quassia amara* (Simurabaceae) on albino rats and mice.] *Rev. Biol. Trop.*, **44-45**, 47-50.

Gillin, F.D., Reiner, D.S. (1982). *In vitro* activity of certain quassinoid anti-tumor agents against *Entamoeba histolytica*. *Arch. Invest. Med.*, **13 (suppl. 3)**, 43-49.

Hall, R.L., Oser, B.L., (1965). Recent progress in the consideration of flavoring ingredients under the Food Additives Amendment. III. GRAS substances. Food Technology, **19,** 151-197.

Lee, K.H. (1999). Novel antitumor agents from higher plants. *Med. Res. Rev.*, **19**, 569-596.

Leskinen, V., Polonsky, J., Bhatnagar, S. (1984). Antifeedant activity of quassinoids. *J. Chem. Ecol.*, **10**, 1497-1507.

Leung, A.Y., Fosters, S.(1996). Quassia. Encyclopedia of Common Natural Ingredients Used in Food, Drugs and Cosmetics. J. Wiley & Sons Inc., pp. 430-431.

Margaria, R. (1963). Analisi dei gruppi lattinici di una quassina greggia. Communication et relation au Comité por l'Etude des Bossions Alcooliques Aromatisées de la Federvini. Milan, Institut de Physiologie de l'Université, pp. 1-10.

Njar, V.C.O., Alao, T.O., Okugun, J.I., Holland, H.L. (1993). 2-Methoxycanthin-6-one: A new alkaloid from the stem wood of *Quassia amara. Planta Med.* **59**, 259-261.

Njar, V.C.O., Alao, T.O., Okugun, J.I., Raji, Y., Bolarinwa, A.F., Nduka, E.U. (1995). Antifertility activity of *Quassia amara*: Quassin inhibits the steroidogenesis in rat leydig cells *in vitro*. *Planta Med*. **61**, 180-182.

Park, M.H., Maeda, M., Komura, H., Nakanishi, K., Nomoto, K. (1987). Acute insecticidal activity of quassin and its congeners against the American cockroach. *Chem. Pharm. Bull.*, **35**, 3082-3085.

Pierré, A., Robert-Géro, M., Tempete, C., Polonsky, J. (1980). Structural requirements of quassinoids for the inhibition of cell transformation. *J. Bichem. Biophys. Res. Commun.*, **93**, 675-686.

Raji, Y., Bolarinwa, A.F. (1997). Antifertility activity of *Quassia amara* in male rats – *in vivo* study. *Life Sci.*, **61**, 1067-1074.

Robins, J.R., Morgan, M.R.A., Rhodes, M.J.C., Furze, J.M. (1984). An enzyme-linked immuno-sorbent assay for quassin and closely related metabolites. *Annal. Biochem*. **136**, 145-156.

Scientific Committee on Food (2001). Guidance on submissions for food additive evaluations. Opinion expressed on 11 July 2001. Available at http://europa.eu.int/comm/food/fs/sc/scf/out98_en.pdf

Trager, W., Polonsky, J. (1981). Antimalarial activity of quassinoids against chloroquine-resistant *Plasmodium falciparum in vitro*. *J. Am. J. Trop. Med. Hyg.*, **30**, 531-537.