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# Opinion of the Scientific Committee on Food on the presence of β-asarone in flavourings and other food ingredients with flavouring properties

(adopted on 12 December 2001)

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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 implications for human health of the presence of β-asarone (2,4,5-trimethoxy-1-propenylbenzene) in the diet.

## Introduction

Previous evaluations

The Scientific Committee for Food considered β-asarone in 1979 (SCF, 1979) and recommended limits of 0.1 mg/kg for foods and beverages with exceptional limits of 1 mg/kg for alcoholic beverages and seasonings used in snack foods (EEC, 1988).

The Council of Europe Committee of Experts on Flavouring Substances (CEFS) evaluated β-asarone as an active principle in food flavourings in 1981. The recommended limits for β-asarone were 0.1 mg/kg for foods and beverages with exceptional limits of 1 mg/kg for alcoholic beverages and foods containing *Acorus calamus* L. or *Asarum europaeum* L. (CoE, 1981). In 1998, the data were reviewed. The CEFS concluded that β-asarone was clearly carcinogenic in rodents and potentially genotoxic and that it would be prudent to reduce the level of β-asarone as far as possible, but there may be a need for specific exceptions for alcoholic beverages. CEFS encouraged the use of *Acorus calamus* varieties with low contents or free of β-asarone and proposed limits of 0.05 mg/kg for foods and beverages and 0.5 mg/kg for alcoholic beverages traditionally flavoured with calamus. The tetraploid

form of *Acorus calamus* should be placed in category 6, i.e. the plant is considered as unfit for human consumption in any amounts. It was further concluded that the exceptional limit of 1 mg/kg for food containing *Acorus calamus* or *Asarum europaeum* could be removed. *In vivo* mutagenicity studies and DNA binding studies were requested in order to clarify whether β-asarone is genotoxic or not (CEFS, 1998).

JECFA has not established an ADI for β-asarone but recommended that the oil of calamus used in foods should have the lowest practicable levels of β-asarone (JECFA, 1981).

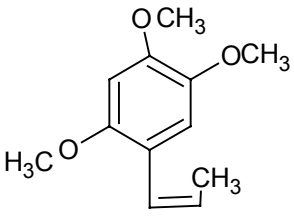
Current Regulations

Annex II of Directive 88/388/EEC on flavourings sets the following maximum levels for β-asarone in foodstuffs and beverages to which flavourings or other food ingredients with flavouring properties have been added: 0.1 mg/kg in foodstuffs and beverages, with the exception of 1 mg/kg in alcoholic beverages and seasonings used in snack foods. β-Asarone as such may not be added to foodstuffs (EEC, 1988).

In the US, calamus oil and its extracts are prohibited from use in food (Federal Register, 1968).

**Chemical characterisation**

Name: β-asarone
Synonyms: cis-asarone
Systematic name: cis-2,4,5-trimethoxy-1-propenylbenzene
CAS no: 5273-86-9
Structure:



**Exposure assessment**

β-Asarone is a flavouring active principle found in the plants *Acorus calamus* (calamus) and *Acorus europaeum* (hazelwort) which have a history of use in herbal medicine (Wren, 1988; Leung, 1980). The volatile oil from the tetraploid "Indian" form of calamus contains β-asarone as a major component (up to 95%) whereas the oil from the "European" or triploid form contains less than 10% β-asarone. A diploid form of the plant contains virtually no β-asarone (Lander and Schreier, 1990) but this is not yet commercially available. No data on the use of hazelwort oil in foodstuffs have been identified.

Calamus oil is largely used in the production of alcoholic beverages such as bitters, liqueurs and vermouths. Levels of up to 0.35 mg/kg have been detected in vermouths (Mérat *et al.*, 1976) and, in a selection of alcoholic drinks (Yabiku and Lajola, 1980) β-asarone concentrations were up to 4.96 mg/kg (the latter exceeding the exceptional limit of 1 mg/kg specified by Council Directive 88/388/EEC). A 1994 survey of 39 herbal liqueurs conducted by the Bavarian State Control Authorities (1994) indicated that 8 samples contained more than 0.5 mg/l β-asarone with 5 of these containing more than 1 mg/l. A 1999 survey of herbal liqueurs reported that only 2/26 contained more than 1 mg/l β-asarone, the remainder contained 0.41 mg/l or less (Bavarian State Control Authorities, 1999).

Calamus oil is used in foods such as frozen desserts, yoghurts, cakes, confectionery and desserts at levels of 0.2, 0.2, 0.3, 0.3 and 3 mg/kg, respectively (Burdock, 1994). This could result in β-asarone levels of 0.014, 0.014, 0.021, 0.021 and 0.21 mg/kg in the final product, assuming that the calamus oil used contains 7% β-asarone. These estimates suggest that some products could exceed the general limit of 0.1 mg/kg specified by Council Directive 88/388/EEC.

Using data from the survey of British adults (Gregory *et al*., 1990) and assuming the concentrations of β-asarone are as given above, intakes of β-asarone range from 0.8-2.3 μg/day from cakes, 11-27 μg/day from desserts, 1.2 to 4.1 μg/day from yoghurts and frozen desserts and 0.4 to 1.4 μg/day in confectionery for mean and high level (97.5 %ile) consumers. Data on β-asarone levels in other food sources are not available.

Using data from the survey of British adults (Gregory *et al*., 1990), mean and high level consumption of liqueurs and vermouth is 18.5 and 80.4 g/day, respectively, and assuming that β-asarone was present at 1 mg/l, intake would be up to 80.4 μg/day.

Based on these limited data, maximum β-asarone intake can be estimated to be about 115 μg/ day. This is about 2 μg/kg bw/day.

## Hazard identification and characterisation

Absorption, distribution, metabolism and excretion

The metabolism of β-asarone has not been established. As a 1-propenylbenzene its metabolism will differ from that of allylbenzenes such as safrole and estragole, which are metabolised via hydroxylation at the 1'-position. Based on its chemical structure, it is likely that β-asarone is demethylated.
In rats, given intra-peritoneal (i.p.) injections of β-asarone, small amounts of ninhydrin-positive substances (believed to be phenylisopropylamines or amphetamines) were detected in the urine with maximum excretion occurring after 24-48 hours (Oswald *et al*., 1969). Similar substances were found in rats treated with myristicin, safrole, isosafrole or trans-(α) asarone.

Toxicity

Where appropriate the toxicity of calamus oil has also been considered in this review.

The following sections contain references to a number of studies reviewed by JECFA (1981). These were part of a package of data submitted by the US Food and Drug Administration which included acute, sub-acute, sub-chronic and chronic studies. The information was a mixture of study reports, pathology reports and memos. Some details are published in the open literature and the references are given where possible. However, the majority of the information is unpublished and has been taken from the JECFA monograph.

Acute toxicity
β-Asarone has an oral $LD_{50}$ of 1010 mg/kg bw in rats and an i.p. $LD_{50}$ of 184 mg/kg bw in mice (JECFA, 1981).
The oral $LD_{50}$ of calamus oil in rats is reported to be 8880 mg/kg bw (cited Opdyke, 1977). Jenner *et al*.,(1964) reported an oral $LD_{50}$ in rats of 777 mg/kg bw for Jammu calamus oil (containing approximately 75% β-asarone; it is stated by JECFA but not by Jenner that Jammu calamus oil was used). Tremors, weight loss and a "scrawny appearance" were noted. JECFA also quotes data from an unpublished study giving oral $LD_{50}$s of 4331 and 3497 mg/kg for Kashmir and European calamus oils, respectively. These contain approximately 5% β-asarone.

Sub-acute toxicity
Pre-weanling Sprague Dawley rats were given i.p. doses of 100 mg/kg bw/day β-asarone or 100 or 250 mg/kg bw/day European calamus oil for 5 days (Ramos-Ocampo and Hsia, 1987). No animals died as a result of treatment. Weight loss and decreased food consumption were reported. Adrenal weights were increased and heart and thymus weights reduced. However, when examined microscopically, pathological

changes were only found in the thymus, where an increase in single cell degeneration in the thymus was observed (in the animals treated with calamus oil, single cell degeneration was seen but thymus weights were unaffected). A range of biochemical and haematological parameters was measured. There was no effect on haematology and no changes in enzyme levels indicating hepatotoxicity.

Sub-chronic toxicity

In an 18 week feeding study, groups of 10 Osborne Mendel rats/sex/dose were fed a diet containing 0, 1000, 2500, 5000 or 10,000 mg/kg Jammu oil of calamus (Taylor *et al*., 1967, Hagan *et al*., 1967). The dose is not given on a body weight basis but can be estimated to be 0, 100, 250, 500 or 1000 mg/kg bw (WHO, 1987). Growth was depressed in all treated groups and mortality was increased at levels of 250 mg/kg bw and above. At the same dose levels, gross liver changes and serous effusions in the abdominal and pleural cavities were observed. Microscopic changes in the liver included variable cell size, with distortion of architecture, capsular thickening, proliferation of bile duct epithelium and portal area fibrosis with haemosiderin deposition. Microscopic changes in the heart included necrosis of the muscle fibres, fibrosis and infiltration with mononuclear cells.

Groups of 11 rats/sex/dose (strain unspecified) were fed 0, 0.27, 1.67 and 5.3% hydroalcoholic extract of European oil of calamus in the diet for 13 weeks (unpublished report discussed by JECFA, 1981). The concentrations in the feed were equivalent to 0, 30, 184 and 583 mg/kg β-asarone. The dose is not given on a body weight basis but can be estimated to be 3, 18.4 or 58.3 mg/kg bw β-asarone (WHO, 1987). An additional group received 0.1% Jammu oil of calamus (710 mg/kg β-asarone, equivalent to 71 mg/kg bw). Growth depression was noted in the Jammu oil of calamus group only. No gross or microscopic effects or effects on clinical chemistry, haematology, urinalysis or organ weights were observed.

The toxicity of different varieties of calamus oil administered in the diet or by gavage was compared (unpublished study discussed by JECFA, 1981). Groups of 10 rats/sex/dose (strain unspecified) were fed 10,000 mg/kg Jammu, European or Kashmir oil of calamus (equivalent to 1000 mg/kg bw) in the diet or were given gavage doses of 250, 847 or 1082 mg/kg bw of the three different calamus oils for 9-14 weeks. In the dietary study, atrophy of cardiac muscle cells was observed in all treatment groups, with cardiac fibrosis being observed in the European and Jammu groups and fatty infiltration in the Jammu calamus group only. Liver toxicity was observed in the dietary study with the most severe effects being present in the Jammu calamus group. Fatty degeneration in the centrilobular region indicated that it was associated with chronic passive hyperaemia. The passive hyperaemia was found in all groups (including controls) but was worse in the Jammu calamus group and was taken to indicate cardiac insufficiency. Coagulative necrosis in the centrilobular region, hepatic fibrosis and bile duct hyperplasia were also apparent in the livers of rats in this

group. Similar effects were seen in the gavage study, being most severe in the Jammu calamus group, followed by the European calamus group.

Chronic toxicity/carcinogenicity

In a 2 year feeding study, an increased incidence of leiomyosarcomas was observed in the small intestines of Osborne-Mendel male rats fed a diet containing either 0.04, 0.08 or 0.2% (400, 800 or 2000 mg/kg) β-asarone or calamus oil equivalent to 0.2% (2000 mg/kg) compared to untreated controls (unpublished study, cited by JECFA, 1981). On a body weight basis the β-asarone dose can be estimated to be approximately 20, 40 or 100 mg/kg bw/day respectively (WHO, 1987). Twenty five animals of each sex per group were treated. The numbers of these tumours reported in the males were 1/25, 6/25 and 9/25 in the 0.04, 0.08 and 0.2% β-asarone groups respectively. A tumour was also found in 1 of the males in the calamus oil group. Reduced survival was apparent in the treated rats (none of the animals receiving 0.2% β-asarone or the calamus oil survived beyond 84 weeks and mortality was also increased in the 0.08% β-asarone group). Gross and microscopic changes observed included cardiac atrophy and fibrosis, fatty degeneration and infiltration of the heart was apparent at all doses but was more severe in the treated animals. Thrombosis within the chambers of the heart occurred in the 0.08 and 0.2% beta-asarone groups and in the calamus oil group. The incidence of hepatic angiectasis and coagulative necrosis tended to increase with dose; serous fluid was observed in the pleural and abdominal cavities. Passive hyperaemia was more notable in the lung, kidneys and liver of treated animals; this was thought to be due to faulty cardiac function. The group size was low (25 animals of each sex per group) and it should be noted that leiomyosarcomas were also found in 2 control females but at different locations to those in the treated animals. Details of the different locations are not given. Leiomyosarcomas are considered to be unusual tumours.

Groups of 25 rats/sex/dose (strain unspecified) were fed 0, 500, 1000, 2500 or 5000 mg/kg Jammu oil of calamus for two years (Taylor *et al*., 1967; with more details provided in JECFA, 1981). The dose is not given on a body weight basis, but can be estimated to be approximately 0, 25, 50, 125 or 250 mg/kg bw/day (WHO 1987). A dose-related increase in mortality occurred with female rats dying earlier than the males in all treatment groups. All animals in the 5000, 2500 and 1000 mg/kg diet, group died within 45, 68 and 104 weeks respectively; it is unclear whether the animals were culled when moribund or found dead. In the males, growth rates were severely reduced at doses of 2500 mg/kg bw and above; at lower doses weight gain was normal for the first 26 weeks but was depressed thereafter. In the females dose-related weight loss occurred at doses of 1000 mg/kg and above; at the low dose, weight gain was normal for the first year and depressed thereafter. Gross macroscopic changes in the liver consisted of discolouration, leathery texture and blunted edges. Degenerative and regenerative changes were observed on microscopic examination. Other gross changes were fluid in the pleural and peritoneal cavities and tumourous masses in the

intestine. The tumours (0/25, 3/25, 0/25, 0/25 and 0/25 in the females and 0/25, 0/25, 5/25, 2/25 and 0/25 in the males in the 0, 500, 1000, 2500 and 5000 mg/kg groups, respectively) were leiomyosarcomas and were described as malignant, highly pleiomorphic and highly anaplastic. The tumours were most common in the duodenum and appeared to have arisen from the musculature of the tunica propria of the mucosae. The incidence of tumours is not dose related but this may have resulted from the high mortality. The histopathological changes observed in the heart and livers of the treated groups were stated to be comparable to those seen in rats given β-asarone (see above). Cardiac atrophy was apparent in all animals but was more severe in the treated groups. Cardiac thrombosis and fatty degeneration and infiltration were observed at doses of 50 mg/kg bw and above. An increase in passive hyperaemia in the liver was found but this was not strongly dose-related. Hepatic nodular hyperplasia was increased in the treated animals but this was not dose-related.

In an additional study by the same workers, groups of 25 rats/sex/dose were fed a diet containing 0, 50, 100 or 5000 mg/kg Jammu oil of calamus for two years (unpublished study cited JECFA, 1981). The dose is not given on a body weight basis, but can be estimated to be approximately 0, 2.5, 5 or 250 mg/kg bw/day (WHO,1987). As in the previous study by the same group, early mortality, heart and liver lesions were observed in the 5000 mg/kg dose group. One leiomyosarcoma was observed in a male in this group.

Groups of 25 rats/sex/dose were fed a diet containing 0, 0.1, 0.5, 1 or 2% (1000, 5000, 10,000 or 20,000 mg/kg) European oil of calamus for two years (unpublished study, cited by JECFA, 1981). The dose is not given on a body weight basis, but can be estimated to be approximately 50, 250, 500 or 1000 mg/kg bw/day (WHO, 1987). Leiomyosarcomas and hepatocellular adenomas and carcinomas were observed in the rats in the 1 and 2% dose groups. Microscopic liver changes included hyperaemia, necrosis, hepatocellular vacuolation and bile duct proliferation. Myocardial atrophy, fibrosis, fatty degeneration and fatty infiltration was found in the heart, severity increasing with dose from 250 mg/kg bw/day. The damage in the heart was considered to be sufficient to account for the congestion and hyperaemia observed in the liver and other organs.

In pre-weanling (12 day old) B6C3F1 mice given either single i.p. injections of 52 mg/kg bw β-asarone or successive injections on days 1, 8, 15 and 22 (total dose approximately 1 mg), an increased incidence of hepatomas was observed (Wiseman *et al*., 1987). A control group was treated with solvent only. After 12 months, 69% of the 43 mice treated with a singles does of β-asarone had hepatomas (1.1+/- 1 per mouse). In mice treated with 4 doses of β-asarone, 83% had hepatomas (2.4 +/- 1.7 per mouse) when autopsied at 13 months.

Genotoxicity

*In vitro*

Both positive and negative results have been reported for β-asarone in the Ames test. Hsia *et al* (1979) found that β-asarone (manufactured in house, but stated to be chromatographically pure by TLC) was not mutagenic in *Salmonella* strains TA98, TA100, TA1525, TA1537 or TA1538 at concentrations of 2, 20 and 200 μg/plate in the presence of S9 activation. In tests conducted by Ramos-Ocampo and Hsia (1988), β-asarone was not found to be mutagenic at concentrations of 0.2 to 200 μg/plate in strains TA97, TA98, TA100, TA1535, TA1537 or TA1538 in either the presence or absence of S9 activation. In a further set of experiments using strain TA100 at concentrations up to 500 μg/plate, β-asarone was not found to be mutagenic. In contrast, Goggelmann and Schimmer (1983) tested commercially purchased β-asarone in strains TA98, TA100, TA1535, TA1537, and TA1538 at concentrations of 10.7 to 1073 μg/plate. The compound was mutagenic in strain TA100 in the presence of S9 only, with a concentration related increase in revertants being apparent. The results are not fully reported but the number of revertants relevant to the control was increased approximately 3, 8 and 20 fold at the 43, 107 and 215 μg/plate concentrations respectively. A range of tinctures, extracts and 3 commercial drugs containing calamus were also tested in strain TA100 in the presence of S9, dose-related increases in revertants were produced by the tincture, extract and one of the drugs. The dose range was based on increasing volumes of the test article, with the β-asarone concentration being unknown.

The studies appear to have been adequately conducted and reported. The reasons for the differing results are uncertain. It has been speculated (CEFS, 1993) that these differences may be due to different experimental procedures being used or differences in the source of the test chemical used.

β-Asarone showed genotoxic effects in the presence but not the absence of S9 in the *Escherichia coli* PQ37 genotoxicity assay (SOS Chromotest) (Kevekordes *et al*., 1999).

Unscheduled DNA synthesis was not detected in isolated rat hepatocytes treated with $10^{-6}$ to $10^{-3}$ M (equivalent to 209 μg/l-209 mg/l and 0.8 to 836 μg/plate) β-asarone (chromatographically pure by TLC) (Ramos-Ocampo and Hsia, 1988); cytotoxicity was apparent at the top dose level. However in more recent studies (Hasheminejad and Caldwell, 1992, 1994) increased UDS was observed in isolated rat hepatocytes treated with β-asarone (original source unclear). The maximum response occurred at a concentration of $5 \times 10^{-4}$ M (equivalent to 104.5 mg/l and 4.18 μg/plate). The effect was abolished by co-treatment with cimetidine suggesting that activation by cytochrome P450 was necessary to achieve the genotoxic effect of β-asarone. Again, the reasons for differing results in the same test system and using the same technique

are uncertain. The methods are reported in detail and the studies appear to have been adequately performed. Scintillation counting was used by both groups but hydroxyurea was included in both sets of experiments to inhibit replicative DNA synthesis.

Clastogenic effects have been observed in human lymphocytes treated with 107 μg/ml β-asarone *in vitro* in the presence of metabolic activation (Abel, 1987). A small increase in sister chromatid exchange was also observed but this was not significant. No effects were found in the absence of metabolic activation. The test article was an isomer mixture containing 70% β-asarone. However, it has been reported that α-asarone is known to induce sister chromatid exchange both in murine bone marrow *in vivo* and in human lymphocytes *in vitro*, in the absence of metabolic activation (Morales-Ramirez *et al*., 1992).

*In vivo*
No data on the *in vivo* genotoxicity of β-asarone have been identified.

Reproductive and developmental toxicity
The vitelinum sac of chicken eggs was injected with 0.04 - 4 mg β-asarone or 0.15 - 15 mg calamus oil. β-Asarone was embryotoxic with 43 and 0 % survival at doses of 0.04 and 4 mg/egg, respectively. No teratogenic effects were apparent (JECFA, 1981).

*Neurotoxicity*
*Acorus calamus* is used in the Ayurvedic medicine system to treat nervous conditions, as a result of this the effects of β-asarone on the central nervous system have been investigated. For example, β-asarone abolished the conditioned avoidance response in 30% of rats given an i.p. 25 mg/kg bw dose (Dandiya and Menon, 1963). The same effects were noted by Sharma *et al*., (1961) though no further details were given. They further reported that the sociability behaviour of cats was reduced by β-asarone and stated that although β-asarone alone did not affect electric shock induced convulsions in rats, it potentiated the convulsing effects of metrazole and picrotoxin; β-asarone alone produced mild myoclonic convulsions.

Sleeping time induced by pentobarbital, hexobarbital and ethanol was lengthened by prior administration of 50 mg/kg β-asarone (Sharma *et al*., 1961). The latter effect may be due to enzyme inhibition rather than neurotoxicity.

In a number of *in vitro* experiments, β-asarone was found to have anti-cholinergic activity and was reported to stop frog hearts in diastole and to reduce the tone and longitude of intestinal movement (Sharma *et al*., 1961).

*Human data*

No data have been identified on the toxicity of β-asarone in humans.

Summary of hazard identification and characterisation

There are few data available on the metabolism of β-asarone.

β-Asarone is of moderate acute toxicity. There are no data on the sub-chronic toxicity of β-asarone, but studies with calamus oil report a range of adverse effects in the heart and liver. Chronic dietary administration of β-asarone and calamus oils, with different amounts of β-asarone, to rats has also been associated with cardiac and hepatic toxicity. The cardiac changes observed included atrophy and fibrosis, fatty degeneration and infiltration. A NOAEL was not determined for the cardiac effects in the rats fed β-asarone, since it was observed in all treated groups, but can be estimated to be 5 mg/kg bw/day for Jammu oil of calamus (equivalent to 3.8 mg/kg bw/day β-asarone). Cardiac failure then resulted in passive hyperaemia in other organs. Necrosis, bile duct proliferation and angiectasis were observed in the liver following chronic treatment. Calamus oils demonstrate similar toxicity to β-asarone, with the toxicity of the oil generally increasing with β-asarone content. A number of effects on the central nervous system have been reported. Few other toxicity data are available.

In a chronic feeding study, β-asarone produced a dose-dependent increase in leiomyosarcomas in the intestine of male rats. The doses tested were high, resulting in severe toxicity and increased mortality. Similar tumours were found in rats treated with toxic doses of calamus oil, a dose response effect was not seen but this may have been masked by poor survival in the higher dose groups. Studies using calamus oil are more difficult to interpret because of the unquantified effects of other components. Liver tumours have been reported in one rat study following the chronic administration of toxic doses of a calamus oil with a low β-asarone content. In a pre-weanling mouse study, where neonatal mice were treated with either a single dose or 4 weekly doses of β-asarone given i.p., an increased incidence of hepatomas was observed after 12 months. However, it is possible that the hepatomas arose from local liver damage. Although no liver tumours were apparent in the rat studies using β-asarone, the numbers used were too small to reliably detect a small carcinogenic effect.

In one report, β-asarone and some preparations of calamus have demonstrated mutagenic activity in the Ames *Salmonella* assay in the presence of exogenous metabolic activation. However, other workers using the same assay have reported negative results. Conflicting results have also been reported for the induction of UDS in rat hepatocytes *in vitro*. Clastogenic effects have been observed in human lymphocytes *in vitro*, however a mixture of α and β-asarone was used. The former compound is known to induce sister chromatid exchange both *in vitro* and *in vivo*.

The mechanism for the carcinogenic effect of β-asarone and calamus oil in the rat is uncertain. An increased incidence of leiomyosarcomas of the intestines was observed in two chronic studies. In one additional study, liver tumours were seen in animals treated with calamus oil with low levels of β-asarone that also resulted in cardiac and liver toxicity. However, the *in vitro* genotoxic potential of β-asarone cannot be discounted.

**Risk characterisation**

The maximum human intake of β-asarone from food and alcoholic beverages can be estimated to be approximately 115 μg/day, i.e. about 2 μg/kg bw/day.
Cardiac atrophy is apparent in rats fed calamus oil containing a dose of β-asarone greater than 3.8 mg/kg bw/day. A NOAEL has not been determined for cardiac effects in rats treated with β-asarone itself.

β-Asarone has shown a weak carcinogenic effect in rats at dose levels of 20 mg/kg bw/day and above. On the basis of the available *in vitro* genotoxicity studies, the genotoxic potential of β-asarone cannot be ruled out. Therefore the existence of a threshold cannot be assumed and the Committee could not establish a safe exposure limit. Consequently, limitations in exposure and use levels are indicated.

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