FOREWORD INTRODUCTION

β-IONONE [(E)-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-3-buten-2-one]

CAS N°: 79-77-6

SIDS Initial Assessment Report

For

SIAM 20

Paris, France, 19 – 22 April 2004

1.	**Chemical Name:**	β-Ionone [(E)-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-3-buten-2-one]
2.	**CAS Number:**	79-77-6
3.	**Sponsor Country:**	Germany Contact Point: BMU (Bundesministerium für Umwelt, Naturschutz und Reaktorsicherheit) Contact person: Prof. Dr. Ulrich Schlottmann Postfach 12 06 29 D- 53048 Bonn
4.	**Shared Partnership with:**	BASF AG, Germany; DSM, Netherlands
5.	**Roles/Responsibilities of the Partners:**	-
•	Name of industry sponsor /consortium	BASF AG, Germany Contact person: Dr. Rolf Sarafin, GUP/CL - Z570 D-67056 Ludwigshafen
•	Process used	see next page
6.	**Sponsorship History**	
•	How was the chemical or category brought into the OECD HPV Chemicals Programme ?	by ICCA-Initiative
7.	**Review Process Prior to the SIAM:**	last literature search (update): 8 October 2004 (Human Health): databases medline, toxline; search profile CAS-No. and special search terms 11 November 2004 (Ecotoxicology): databases CA, biosis; search profile CAS-No. and special search terms OECD/ICCA
8.	**Quality check process:**	IUCLID was used as a basis for the SIDS dossier. All data were checked and validated by BUA. A final evaluation of the human health part has been performed by the Federal Institute for Risk Assessment (BfR) and of the ecotoxicological part by the Federal Environment Agency (UBA).
9.	**Date of Submission:**	Deadline for circulation: 21 January 2005
10.	**Date of last Update:**	last literature search (update) of sponsor company: 3 June 2004 (Human Health): databases medline, toxline; search profile CAS-No. and special search terms; 3 June 2004 (Ecotoxicology): databases CA, biosis; search profile CAS-No. and special search terms; 4 October 2004 (Ecotoxicology): database CA registry, search profile CAS-No. with special search terms, Beilstein

11. Comments:

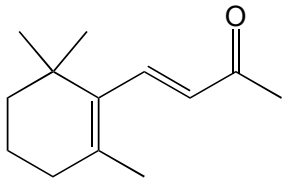
OECD/ICCA - The BUA* Peer Review Process

Qualified BUA personnel (toxicologists, ecotoxicologists) perform a quality control on the full SIDS dossier submitted by industry. This quality control process follows internal BUA guidelines/instructions for the OECD/ICCA peer review process and includes:

- a full (or update) literature search to verify completeness of data provided by industry in the IUCLID/HEDSET
- Review of data and assessment of the quality of data
- Review of data evaluation
- Check of adequacy of selection process for key studies for OECD endpoints, and, where relevant, for non-OECD endpoints by checking original reports/publications
- Review of key study description according robust summaries requirements; completeness and correctness is checked against original reports/publications
  (if original reports are missing: reliability (4), i.e. reliability not assignable)
- Review o f validity of structure-activity relationships
- Review of full SIDS dossier (including SIAR, SIAP and proposal for conclusion and recommendation for further work)
- In case of data gaps, review of testing plan or rationale for not testing

* BUA (GDCh-Beratergremium für Altstoffe): Advisory Committee on Existing Chemicals of the Association of German Chemists (GDCh)

SIDS INITIAL ASSESSMENT PROFILE

CAS No.	79-77-6
Chemical Name	-Ionone [(E)-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-3-buten-2-one]
Structural Formula	

SUMMARY CONCLUSIONS OF THE SIAR

Human Health

From animal experiments it can be concluded that -ionone is absorbed after oral exposure. Metabolism takes place mainly in the liver. Metabolites, which were identified in the urine of exposed rabbits, are 3-oxo- -ionone, 3-oxo- -ionol, dihydro-3-oxo- -ionol and 3-hydroxy- -ionol. -Ionone was found to be an inducer of CYP 1A and 2B isozymes in the liver of rodents.

-Ionone has only low acute toxicity after oral ingestion. A gavage study conducted with a mixture of 60 % -Ionone and 40 % -ionone revealed a LD_{50} of 4590 mg/kg bw. Clinical signs of toxicity were depression and tremors.

In studies conducted according to OECD test guidelines and under GLP conditions, -ionone was not irritating to the skin of rabbits after semiocclusive application for 4 hours and only slightly irritating to the eyes. After a 24-hours exposure under occlusive conditions, a slight irritation of the skin was observed in rabbits. A limited human patch test did not reveal a potential for skin irritation when a not further specified mixture of - and -ionone was applied undiluted to the skin of volunteers.

A limited Guinea pig maximization test found no evidence that -ionone is a dermal sensitizer. According to secondary sources, ionone (a not further specified mixture of - and -ionone) was negative in an open epicutaneous test with Guinea pigs as well as in a human maximization test with a product containing 97.5 % -ionone and 2.5 % -ionone.

The administration of -ionone over a period of 90 days according to OECD TG 408 at dietary concentrations of 100, 1000 and 10 000 ppm (7 and 8 mg/kg bw/day, 72 and 83 mg/kg bw/day or 720 and 801 mg/kg bw/day for males and females) to rats led to signs of general systemic toxicity at the high and mid dose. Target organs were liver, kidneys and thyroid glands. The liver findings in both sexes and the increased kidney weights in high dose females were indicative of adaptive and most likely reversible processes with the aim to increase the metabolizing and/or excretory capacity of these organs. The findings in males with respect to kidneys as well as kidney relevant parameters should be seen in the light of high amounts of alpha2u-globulin in these animals. The occurrence of alpha2u-globulin was confirmed by immunohistochemical examination. The accumulation of this protein appears to be a unique feature of male rats and is not known to occur in other species, including man. No signs of neurotoxicity were observed during functional observational battery as well as measurement of motor activity performed towards the end of the administration period.
Thus, the no-observed-effect-level (NOEL) under the conditions of the present study was 100 ppm for both sexes (about 7 and 8 mg/kg bw/day for males and females) based on adaptive liver effects in both sexes and minor urine findings in males at 1000 ppm which correspond to a dosage of 72 and 83 mg/kg bw/day for males and females (no-observed-adverse-effect-level, NOAEL). The lowest-observed-adverse-effect-level (LOAEL) was found at 10 000 ppm (720 and 801 mg/kg bw/day for males and females) due to liver, kidney and thyroid findings in both sexes.

-Ionone gave no indication of a mutagenic effect in bacteria or a clastogenic potential in an *in vivo* mouse micronucleus test. Therefore, there is no indication of a genotoxic potential *in vivo*.

No studies that would be considered adequate for the evaluation of carcinogenic potential were available. A short-term screening experiment investigating a tumor-promoting potential on mouse skin did not indicate such an effect at a low test concentration.

In a well-conducted 90 days study in rats according to OECD TG 408 with administration of the test substance in the diet, -ionone did not have the potential to damage the reproductive organs at least up to the highest tested concentration of 10,000 ppm (720 and 801 mg/kg bw/day for males and females).

Based on the results of a GLP and guideline conforming developmental toxicity study (OECD TG 414) with gavage application of -ionone, the no observed adverse effect level (NOAEL) for maternal toxicity was 100 mg/kg bw/day. The NOAEL for prenatal developmental toxicity could be fixed at the highest tested dose (400 mg/kg bw/day). The test substance had no influence on gestational parameters and induced no adverse signs of developmental toxicity and in particular no indications of teratogenic effects up to and including the highest dose level were observed.

Environment

The colorless to yellowish liquid -ionone has a water solubility of about 0.169 g/l and a vapor pressure of about 0.009 hPa at 25 °C. The measured log K_{OW} of 4.0 at 25 °C, the calculated log K_{OC} of 2.80 - 3.34 and the calculated BCF of 501 indicate a potential for bio- and geoaccumulation. According to distribution modeling using Mackay Level I, water (34 %), soil (27 %), sediment (27 %) and air (12 %) are the main targets for the compound. -Ionone is with > 70 % (within 28 days, 10-day-window criteria fulfilled) readily biodegradable according to OECD criteria. In the atmosphere, it will be rapidly photodegraded by reactions with OH radicals (calculated $t_{1/2}$: 1.6 hours) and ozone (calculated $t_{1/2}$: 18 minutes).

Results on acute aquatic toxicity are available for fish (*Pimephales promelas*; LC_{50} (96 hours): 5.1 mg/l), invertebrates (*Daphnia magna*; EC_{50} (48 hours): 3.7 mg/l) and algae (*Scenedesmus subspicatus*; $E\mu C_{50}$ (72 hours): 22.2 mg/l; EbC_{50} (72 hours): 21.2 mg/l). Based on these acute toxicity studies, -ionone is considered as toxic to aquatic organisms. No results on prolonged or chronic toxicity to aquatic organisms are available. According to the EU risk assessment procedure, a $PNEC_{aqua}$ of 3.7 µg/l was obtained by applying an assessment factor of 1000 on the lowest $L(E)C_{50}$ value, the result of the test with *Daphnia magna*.

Exposure

In the year 2003 the world production of industrial -ionone was between 4000 and 8000 tonnes/a and in Europe between 1000 and 5000 tonnes/a. In the Sponsor country the production volume of the sole producer is between 1000 and 3000 tonnes/a. The production in USA and Asia was < 1000 tonnes/a and < 2000 tonnes/a, respectively.

In the Sponsor country about 70 % of the manufactured industrial -ionone is used as intermediate internally for complete consumption in chemical processes for the synthesis of fine chemicals (e.g. vitamins, aroma chemicals). About 30 % of -ionone are distributed to industrial clients which are using the substance as intermediate for chemical syntheses or directly as flavoring compound and/or aroma additive in e.g. food.

Exposure to β-Ionone occurs via food, cosmetics and some house wares like cleaning agents (flavoring compound, aroma additive). In cosmetics usual concentrations are up to 0.3 % and in food maximum amounts added ranging from 0.5 – 10 ppm. β-ionone occurs also naturally in food (some plants e.g. corn) and plant extracts used for example in perfumes. Typical use concentrations in final products are 0.03 % (soap), 0.003 % (detergent), 0.016 % (creams, lotions) and 0.3 % (perfume). β-Ionone is listed in the Danish, Norwegian and Swedish product register and not listed in the Swiss product register. For Denmark and Norway the use in consumer preparations (Denmark: cleaning / washing agent) is stated.

The substance naturally occurs as a biogenic volatile organic compound and shows a ubiquitous occurrence in the air due to emissions from plants or surface waters. For instance, β-Ionone was found in concentrations ranging from 0.002 µg/l up to 1.2 µg/l in waters of lakes and rivers mainly due to biotransformation processes in phytoplankton. Further, it was measured in red wine with 0.72 µg/l and was described as a volatile compound in

beef flavor. It was also identified in several fruits as well as in drinking water- and in wastewater samples. The odor threshold is indicated with 0.007 ppb or 56 ng/m³ based on vapor.

In the Sponsor country (Germany) worker protection is adequate and includes the use of appropriate technical equipment during substance handling and the use of protective equipment, etc.

RECOMMENDATION AND RATIONALE FOR THE RECOMMENDATION AND NATURE OF FURTHER WORK RECOMMENDED

Human Health: The only hazards identified are slight irritation to the eyes and changes in the liver, kidneys and thyroid after repeated oral exposure, which were either of minor severity or were considered to be a species-specific effect in male rats. Given the main use as a chemical intermediate and the low content of the substance in consumer products in the Sponsor country, the chemical is currently of low priority for further work. Countries may desire to investigate any exposure scenarios that were not presented by the Sponsor country.

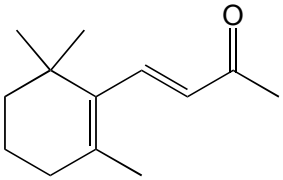
Environment: -Ionone possesses properties indicating a hazard for the environment. Based on the data presented by the Sponsor country (relating to production by one producer which accounts for approx. 10 – 40 % of global production and relating to the use in several OECD countries), exposure to the environment from human production and use of -ionone is anticipated to be low, and therefore this chemical is currently of low priority for further work. Countries may desire to investigate any exposure scenarios that were not presented by the Sponsor country.

SIDS Initial Assessment Report

1 IDENTITY

1.1 Identification of the Substance

CAS Number: 79-77-6
IUPAC Name: (E)-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-3-buten-2-one
Molecular Formula: $C_{13}H_{20}O$
Structural Formula:



Molecular Weight: 192.3 g/mole
Synonyms: 3-Buten-2-one, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-, (E)-
(E)-Beta-ionone
trans-β-Ionone
β-Ionone

The C_{13} ketone β-Ionone has a cyclic terpenoid skeleton and occurs in many essential oils (Ullmann's Encyclopedia, 2000).

There are two CAS Numbers available:

a) CAS: 14901-07-6 for 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-3-buten-2-one which does not discriminate between the Z and E-Isomer and

b) CAS: 8013-90-9 for the mixture of alpha- and beta-Ionone.

In the Sponsor country only the E-Isomer of β-ionone is produced and marketed (see also chapter 1.2). The literature searches were performed with CAS number 79-77-6 (E-isomer).

1.2 Purity/Impurities/Additives

The purity of the (E)-β-ionone BASF product is at ≥ 97 % determined by gas chromatography. It does not contain (Z)-β-ionone, according to ^{1}H-NMR. Impurities comprise mainly other ionone isomers like α-ionone (127-41-3), sum according to the specifications ≤ 3 % (w/w). The BASF AG β-ionone product contains no additives (BASF AG, 2004a).

The DSM (Netherlands) also produces only the (E)-β-ionone – former owners and producers were Roche and Givaudan -, according to ^{1}H-NMR (BASF AG, 2004b).

1.3 Physico-Chemical properties

Table 1 Summary of physico-chemical properties

Property	Value	Comment; Reference
Physical state	Colorless to yellowish liquid	BASF AG, 2003a
Melting point	-35 °C	Beilstein, Handbook (online)
Boiling point	267 °C at 1013 hPa 262.93°C (pressure not indicated)	BASF AG, 2004c calculated via MPBPWIN v1.41; BASF AG, 2005
Relative density	0.9447 g/cm³ at 20 °C	measured; Baglay, Gurarly and Kuleshov, 1988
Vapor pressure	0.009 hPa at 25 °C 0.017 hPa at 25 °C	extrapolation of measured data; BASF AG, 1989a calculated via MPBPWIN v1.41; BASF AG, 2005
Water solubility	0.169 g/l at 25 °C	measured; Fichan, Larroche and Gros, 1999
Partition coefficient n-octanol/water (log value)	4.0 at 25 °C	BASF AG, 1989b (measured)
Henry's law constant	1.023 Pa*m³/mol at 25 °C 17.63 Pa*m³/mol at 25 °C	calculated based on measured data for water solubility and vapor pressure; Thomas, 1982 calculated; BASF AG, 2003b

With 0.9447 g/cm³ the relative density of β-ionone is slightly lower than that of water (Baglay, Gurarly and Kuleshov, 1988). Henry's law constant values of 1.023 Pa*m³/mol and 17.63 Pa*m³/mol at 25 °C indicates moderate volatility of β-ionone according to Thomas (1982).

The structure of β-ionone does not indicate explosive or oxidative properties.

2 GENERAL INFORMATION ON EXPOSURE

2.1 Production Volumes and Use Pattern

In the year 2003 the world production of industrial β-ionone was between 4000 and 8000 tonnes/a and in Europe between 1000 and 5000 tonnes/a (BASF AG, 2004d). The production in USA and Asia was < 1000 tonnes/a and < 2000 tonnes/a, respectively.

In Germany the production volume of the sole producer is between 1000 and 3000 tonnes/a (BASF AG, 2004d).

In Europe industrial β-ionone is exclusively manufactured in dedicated, closed systems (BASF AG, 2004d). Several routes of synthesis are described: Ionones, irones, and methylionones, as well as allylionone, are all produced by analogous routes. Special procedures must be used to obtain a particular isomer, either pure or as the main component. β-Ionone can be made from citral and acetone or from dehydrolinalool and diketene or isopropenyl methyl ether via pseudoionone (Ullmann's Encyclopedia, 2000).

In the Sponsor country about 70 % of the manufactured industrial β-ionone is used as intermediate internally for complete consumption in chemical processes for the synthesis of fine chemicals (e.g.

vitamins, aroma chemicals). About 30 % of β-ionone are distributed to industrial clients which are using the substance as intermediate for chemical syntheses or directly as flavoring compound and/or aroma additive in e.g. food (BASF AG, 2004d).

Other known producers of β-ionone are in Europe DSM (Netherland), and in Asia Pirmal (India) and Kuraray (Japan), respectively (BASF AG, 2004d).

In the Sponsor country worker protection is adequate and includes the use of appropriate technical equipment during substance handling and the use of protective equipment, etc. However, the risk of exposure to β-ionone may exist after spillages and during accidental exposure. Likewise dermal contact to the pure substance may result only from accidental exposure since the majority of the material (70 %) is used as an intermediate in closed systems, and small quantities (ca. 30 %) are filled and distributed to industrial clients (BASF AG, 2004d).

β-Ionone is listed in the Danish, Norwegian and Swedish product register and not listed in the Swiss product register (Swiss product register, 2004). For Denmark and Norway the use in consumer preparations (Denmark: cleaning / washing agent) is stated (SPIN, 2004).

2.2 Environmental Exposure and Fate

2.2.1 Sources of Environmental Exposure

Due to the closed production system at BASF plant sites, exposure of the environment at the BASF plant site in Ludwigshafen (Germany) to β-ionone is limited to maintenance and filling transfer operations.

During production and internal processing at BASF AG Ludwigshafen less than 25 kg/a were emitted into the air in the year 2000 (BASF AG, 2004e). Emission data from other production and processing sites are not available. Data on β-ionone concentrations in the influent or effluent of a wastewater treatment plant are also not available.

Due to its generally natural occurrence β-ionone can be detected in food and in the water compartment. Further, numerous plants contain β-ionone as a general literature search in chemical abstracts showed. Since 1995 there were about 125 publications cited, reporting the occurrence of the substance in plants.

In red wine 0.72 µg/l β-ionone was measured (Ferreira et al., 1998). From the medial zone of maize kernels about 15 ng/h of β-ionone were emitted (Zeringue, 1997). β-Ionone is also described as a volatile in beef flavor (Shahidi, Rubin and D'Souza, 1986).

Further, β-ionone occurs in different types of plants, e.g. tobacco (Salt, Tuzun and Kuc, 1986), the rutacean *Boronia megastigma* (Roberts and Menary, 1994; MacTavish and Menary, 1997 and 2000a and b; Plummer et al., 1998; Plummer, Wann and Spadek, 1999), the caryophyllacean *Cerastium candidissimum* (Lazari, Skaltsa and Constantinidis, 2000), the cistacean *Cistus monspeliensis* (Angelopoulou et al., 2001), the brassicacean *Lepidium meyenii* (Tellez et al., 2002), the fabacean *Medicago marina* (Flamini et al., 2003), the oleacean *Osmanthus fragrans* (Ômura, Honda and Hayashi, 2000), the rosacean *Rosa bourboniana* (Anonymous, 2001) and the labiatean *Thymus serpyllum* (Puri et al., 1985). In different algae species β-ionone was also detected (Moehren und Juettner, 1983; Evans, 1994; Cotsaris et al., 1995; Walsh, Jones and Dunstan, 1998).

β-Ionone was also found in concentrations ranging from 0.002 µg/l up to 1.2 µg/l in waters of lakes and rivers mainly as a result of biotransformation processes in phytoplankton species (Juettner, 1984 and 1992) with a detection limit ranging between 0.001 µg/l (Chorus et al., 1992) and 0.005 µg/l (estimated from graph; Jones and Korth, 1995).

The odor threshold is indicated with 0.007 ppb (56 ng/m^3) in air (Roempp, 2004) and with a water concentration of 1 µg/l β-ionone (subjectively determined (Wilkesmann et al., 1995).

2.2.2 Photodegradation

In the air, β-ionone will be rapidly degraded as indicated by the calculated half life ($t_{1/2}$) of about 1.6 hours for the reaction with OH-radicals ($5 \cdot 10^5$ molecules/cm^3) and about 18 minutes for reaction with ozone molecules ($7 \cdot 10^{11}$ molecules/cm^3) using SRC AOP v1.90 (BASF AG, 2003c, d).

2.2.3 Stability in Water

No data on stability of β-ionone in water are available, but due to the chemical structure a hydrolysis reaction is not expected (BASF AG, 2004f).

2.2.4 Transport between Environmental Compartments

According to distribution modeling using the dynamic Mackay Level I (v2.11), water (34 %), soil (27 %), sediment (27 %) and air (12 %) will be the main targets of β-ionone. Less than 1 % of the substance will be distributed to suspended solids, fish and aerosol (BASF AG, 2004g).

The estimated log K_{OC}-values of 2.80 using SRC's PCKOCWIN v.1.66 (BASF AG, 2003e) and 3.34 (according to Sabljic and Guesten, 1995; cited in the TGD, 2003) indicate a potential for significant adsorption to soil, sediments and suspended solids.

2.2.5 Biodegradation

According to OECD criteria, β-ionone is readily biodegradable and two valid studies are available: In a biodegradation study using the manometric respirometry test (OECD 301F), the biodegradation rate was 79 % of the theoretical oxygen demand (ThOD) within 28 days and the 10-day window criteria was fulfilled (BASF AG, 1989c). In a study according to ISO 14593 (CO_2 – Evolution) degradation, by means of mineralization, of 73 % within 28 days was observed and the 10-day window was fulfilled (RIFM, 2000).

In addition, in a biodegradation test on stability in water using spiked river water, only 5 % of the β-ionone starting concentration (6 µg/l) was detected after 20 hours incubation at 22°C under normal light conditions (Korth, Ellis and Bowmer, 1992).

2.2.6 Bioaccumulation

No experimental data on bioaccumulation of β-ionone are available. Using the equation developed by Veith et al. (1979) recommended in the TGD (2003) and the measured log K_{OW} of 4.0, a bioconcentration factor (BCF) of 501 was calculated.

2.2.7 Other Information on Environmental Fate

No other information concerning the environmental fate of β-ionone is available.

2.3 Human Exposure

2.3.1 Occupational Exposure

No data on human workplace exposure are available.

Exposure may occur during manufacture, transportation and industrial use. The likely primary routes of human exposure to β-ionone are skin contact and inhalation at the work place. Worker exposure in the Sponsor country is limited by enclosed systems, industrial hygiene controls and personal protective measures (protective gloves, safety glasses with side-shields, respiratory protection if ventilation is inadequate).

2.3.2 Consumer Exposure

Consumer is exposed to β-Ionone in food, cosmetics and some house wares like cleaning agents (flavoring compound, aroma additive). In general, consumer exposure is low since only small amounts of β-ionone are contained in cosmetics at usual concentrations of up to 0.3 % and in food in maximum amounts ranging from 0.5 – 10 ppm. β-Ionone was granted GRAS status by FEMA (in 1965) and is approved by the FDA for food use (21 CFR 121.1164) (cited in Opdyke, 1979). The council of Europe (EU, 1999) listed β-ionone giving ADI of 0.1 mg/kg bw/day. The Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2004) has published a monograph and specification, giving conditional ADI of 0 - 0.1 mg/kg bw/day. The Canadian Food Inspection Agency (Feed Section) listed β-ionone as approved feed ingredient with a maximum limit of 12.5 ppm (CFIA, 2004). The Japan Flavor & Fragrance Material Association put β-ionone on their list of designated additives-flavorings as a flavoring used for food in Japan without any details of permitted uptake levels (FFCR Japan, 2004).

According to Opdyke (1979) typical use concentrations in final products are 0.03 % (soap), 0.003 % (detergent), 0.016 % (creams, lotions) and 0.3 % (perfume).

The odor threshold is indicated with 0.007 ppb or 56 ng/m³ based on vapor (Roempp, 2004) and 1 µg/l based on the concentration in water (subjectively determined (Wilkesmann et al., 1995)).

3 HUMAN HEALTH HAZARDS

3.1 Effects on Human Health

3.1.1 Toxicokinetics, Metabolism and Distribution

A few older studies dealing with the metabolic conversion of β-ionone are available. Although they were not conducted according to current standards and under GLP conditions, the results of these studies are considered to give basic and reliable information on this endpoint.

Studies in Animals

In vivo Studies

Urine was collected daily from one male rabbit administered a total of 23 g β-ionone by gavage over 7 days at approximately 1000 mg/kg bw/day and for 4 days after the final dose (Ide and Toki, 1970). 3-Oxo-β-ionone, 3-oxo-β-ionol, dihydro-3-oxo-β-ionol and 3-hydroxy-β-ionol were identified as well as unchanged β-ionone and the glucuronic acid conjugates of 3-oxo-beta-ionol and dihydro-3-oxo-beta-ionol.

The urinary metabolites of two rabbits that were fed a total of 100 g β-ionone for a period of 18 days (daily dose about 2.8 g/animal) were reported as 4-oxo-β-ionone and 4-oxo-β-Ionole (Prelog and Meier, 1950) [Remark: the authors used a different naming system as Ide and Toki, 1970].

A number of studies investigated the potential of β-ionone to induce liver enzymes. In summary, it can be concluded that β-ionone is a potent inducer of cytochrome P450 1A and 2B isozymes in the liver of rodents after oral and subcutaneous treatment (Jeong et al., 1995, 1998 and 1999; Gu et al., 1997; Jeong et al., 2002). These findings are in line with results from a recently conducted subchronic oral toxicity study in rats (BASF AG, 2004h; see chapter 3.1.5). In this study, the increase of liver weights and the hypertrophy of hepatocytes at oral dosages of about 70 - 80 and 700 - 800 mg/kg bw/day was interpreted as a physiological adaptation mechanism (enzyme induction) due to the high intake of the test substance.

Conclusion

From animal experiments, it can be concluded that β-ionone is absorbed after oral exposure. Metabolism takes mainly place in the liver. Metabolites, which were identified in the urine of exposed rabbits, are 3-oxo-β-ionone, 3-oxo-β-ionol, dihydro-3-oxo-β-ionol, 3-hydroxy-β-ionol and glucuronic acid conjugates. β-Ionone was found to be a an inducer of CYP 1A and 2B isozymes in the liver of rodents.

3.1.2 Acute Toxicity

No guideline and GLP conforming acute toxicity study with β-ionone could be located. However, an older but reliable investigation conducted with a mixture of α- and β-ionone is appropriate for the assessment of the acute oral toxicity. It is expected that the acute oral toxicities for the two isomers are similar and therefore the LD_{50} for the mixture is considered to be representative of that for β-ionone.

Studies in Animals

Oral

Groups of 10 male and female Osborne-Mendel rats received a mixture consisting of 60 % α-ionone and 40 % β-ionone by gavage application (Jenner et al., 1964). After a post observation period of 14 days the LD_{50} was calculated to be 4590 mg/kg bw. The times of death were 4 hours up to 4 days after administration of the test substance. As clinical signs of toxicity, depression and tremors were reported. It is not mentioned in the publication if a necropsy was performed.

The low acute toxicity of β-ionone after oral exposure can further be substantiated by a subacute study when rats received 5 consecutive gavage applications of β-ionone (Hoffmann LaRoche Inc., 1975). No mortality was observed up to a dosage of 2000 mg/kg bw, but higher doses showed lethality. As a clinical sign of toxicity, sedation was described at dosages of 500 mg/kg bw or higher. After a post observation period of 15 days, a LD_{50} of 4110 mg/kg bw could be calculated.

Animal studies on acute inhalation and dermal toxicity are not available.

Conclusion

β-Ionone has only low toxicity after acute oral ingestion. A study with gavage application of a mixture of 60 % α-Ionone and 40 % β-ionone revealed a LD50 of 4590 mg/kg bw. Clinical signs were depression and tremors.

3.1.3 Irritation

For assessment of the skin and eye irritation potential, two guideline studies conducted in rabbits as well as two older Draize tests are available. In addition, two limited human patch tests have been performed.

Skin Irritation

Studies in Animals

β-Ionone was not irritating to the skin of 3 rabbits after semiocclusive application for 4 hours in a study according to OECD TG 404 and conducted under GLP conditions (BASF AG, 1992a). Scores for erythema and edema were 0 at all observation times.

In an older Draize test with 3 rabbits, β-ionone showed slight skin irritation after occlusive application of the undiluted substance for 24 hours (Hoffmann-LaRoche, 1967).

Studies in Humans

A limited patch test using undiluted ionone (mixture of α- and β-ionone without further isomer specification) did not produce any reactions in 11 subjects when applied at full strength for 24 hours (Katz, 1946). Limited patch tests with three ionones (α-ionone, methyl ionone and extra pure ionone, without further specification) in 20 patients with different dermatoses elicited negative reactions in all subjects although two persons had erythematous reactions which disappeared within 48 hours (Mendelsohn, 1946).

Eye Irritation

Studies in Animals

In a test with 3 rabbits according to OECD TG 405 and conducted under GLP conditions, slight corneal opacity and iritis was observed after 24 hours of application and conjunctival irritation after 1, 24 and 48 hours (BASF AG, 1992b). All signs of irritation (including corneal opacity) were completely reversible within 72 hours of observation.

An older Draize test with β-ionone (nature of test material not further specified) confirmed the low eye irritation potential of β-ionone (Hoffmann-LaRoche, 1967). After application of 0.1 ml of the undiluted substance into the conjunctival sac of 3 rabbits only slight conjunctival redness was observed shortly after treatment. This finding was completely reversible after 24 hours.

Conclusion

In studies conducted according to OECD test guidelines and under GLP conditions, β-ionone was not irritating to the skin of rabbits after semiocclusive application for 4 hours and only slightly irritating to the eyes. After a 24-hours exposure under occlusive conditions, a slight irritation of the skin was observed in rabbits. A limited human patch test did not reveal a potential for skin irritation when a not further specified mixture of α- and β-ionone was applied undiluted to the skin of volunteers.

3.1.4 Sensitisation

A Guinea pig maximisation test conducted with β-ionone is available as well as secondary citations for an open epicutaneous test in Guinea pigs and a human maximisation test. The two latter studies were conducted with mixtures of α- and β-ionone.

Studies in Animals

Skin

A limited Guinea pig maximisation test was conducted with β-ionone (CAS-no. 14901-07-6)(Takasago International Corporation, 1999). The study is available as English translation of the original report in Japanese language. Although the test has limitations with regard to methodology (reduced animal number, i.e. 5 in test groups, 4 in control group) and documentation, the study is regarded to provide reliable information on this endpoint.

An intradermal induction at a 10 % test concentration in Freund's Complete Adjuvant (FCA) was performed in the test animals. For the second, topical induction the substance was applied at a 10 % concentration in FCA. For the topical challenges, the test concentrations ranged from 5 to 40 % in acetone.

No evidence of erythema or edema was noted in any test group after challenging as well as in the control animals. Sensitisation index (total scores for erythema and edema divided by number of animals) was 0.0 for all groups.

An open epicutaneous test in Guinea pigs was conducted with ionone (mixture of α- and β-ionone) (Klecak, 1985). The results of this study are available as secondary citation from a collection of data only and no further test substance specification was provided. The induction phase consisted of 21 daily open applications to the shaved flank of at least 6 guinea pigs per group. Open challenge applications were made on days 21 and 35. Reactions were read 24, 48 and 72 hours after challenge. A substance was regarded allergenic if at least 1 of 6 animals of the respective concentration group showed positive results when non-irritating concentrations were used for challenge.

Ionone at a concentration of 8 % did not indicate a skin sensitising potential under the test conditions.

Studies in Humans

Skin

Ionone (a mixture of 97.5 % α-ionone and 2.5 % β-ionone) was investigated in the human maximisation test at a concentration of 8 % (Greif, 1967). None of the 25 volunteers showed a positive reaction. Ionone was classified as a non-sensitiser under the conditions of the study.

Conclusion

A limited Guinea pig maximisation test found no evidence that β-ionone is a dermal sensitiser. According to secondary sources, ionone (a not further specified mixture of α- and β-ionone) was negative in an open epicutaneous test with Guinea pigs as well as in a human maximisation test with a product containing 97.5 % α-ionone and 2.5 % β-ionone.

3.1.5 Repeated Dose Toxicity

Several studies with repeated application of β-ionone have been reported. Most of them do not fulfill current guideline requirements and suffer from insufficient documentation of methods and results. However, a recently and well conducted, subchronic study with rats is regarded as appropriate for assessment of this endpoint.

Studies in Animals

Oral

A subchronic oral toxicity study was recently conducted with β-ionone (purity 97.8%) under GLP conditions and according to OECD TG 408 (BASF AG, 2004h). The scope of examinations was extended to cover also effects on reproductive organs (see also chapter 3.1.8).

The compound was administered to groups of 10 male and 10 female Wistar rats at dietary concentrations of 0, 100, 1,000 and 10,000 ppm for 3 months. These concentrations corresponded to dosages of about 7 and 8 mg/kg bw/day, 72 and 83 mg/kg bw/day or 720 and 801 mg/kg bw/day for males and females, respectively. Food consumption and body weight were determined weekly. The animals were examined for signs of toxicity or mortality at least once a day. Detailed clinical examinations in an open field were conducted prior to the start of the administration period and weekly thereafter. A functional observational battery (FOB) and measurement of motor activity was carried out after 13 weeks of treatment. Clinicochemical and hematological examinations as well as urinalyses were performed towards the end of the administration period. Ophthalmological examinations were performed before and towards the end of the administration period. Vaginal smears for estrous cycle determination of all female animals were prepared and evaluated each day during the last 4 weeks of the study. Finally, all animals were assessed by gross pathology, followed by histopathological examinations. Reduced body weights in high-dose dose males were attributed to reduced food intake and considered incidental, or did not achieve statistical significance. In summary, the following substance-related findings were also obtained:

10 000 ppm (720 mg/kg bw/day in males; 801 mg/kg bw/day in females)

- Increased γ-glutamyltransferase, calcium, total protein, albumin, globulins, cholesterol
- Increased amount of urinary ketones and increased number of urinary transitional epithelial cells in both sexes, as well as increased urinary casts in males
- Shortened prothrombin times and increased urinary urobilinogen in females
- Decreased thyroxine in males (- 19 %)
- Significant increase of the absolute and relative liver weights in males and females (absolute weights: + 65 % and + 53 %, relative weights: + 73 % and + 55 %)
- Significant increase of the absolute and relative kidney weights in males (+ 23 % and + 28 %) and of the relative kidney weight in females (+ 11 %)
- Central hypertrophy of hepatocytes in the liver of all males and females
- Peripheral hypertrophy of hepatocytes in the liver of two males
- High incidence of chronic nephropathy in males
- Higher amounts of alpha2u-globuline in tubular epithelial cells of the kidneys in males
- Higher degrees of severity of altered colloid in the thyroid gland of males and high incidence with higher gradings of altered colloid in females

1000 ppm (72 mg/kg bw/day in males; 83 mg/kg bw/day in females)

- Increased urinary casts and urinary transitional epithelial cells in males
- Significant increase of the relative liver weight in males (+ 9 %) and of the absolute and relative liver weights in females (+ 10 % and + 8 %)
- Central hypertrophy of hepatocytes in the liver of 3 males
- Higher amounts of alpha2u-globuline in tubular epithelial cells of the kidneys in males

100 ppm (7 mg/kg bw/d in males; 8 mg/kg bw/d in females)

- No substance-related findings

These observations are briefly discussed in the following:

Concerning clinical examinations, no substance-related findings were obtained. Moreover, no signs of neurotoxicity were observed during functional observational battery as well as measurement of motor activity, performe towards the end of the administration period. The ophthalmological examinations and the estrous cycle determination did not reveal any treatment related effects.

Regarding clinical pathology, marked increases in γ-glutamyltransferase activities and higher total protein, albumin, globulin and cholesterol concentrations were found in high dose animals of either sex and shortened prothrombin times were observed in females of the high dose group. These alterations were considered to be treatment-related and are indicative of hepatic changes primarily caused by microsomal enzyme induction. In clinical chemistry, hepatic microsomal enzyme induction in the rat is characterized by stimulation of the synthesis of γ-glutamyltransferase, clotting factors and cholesterol. Consistent with these effects of hepatic microsomal enzyme induction are the increased liver weights and the literature findings on CYP 450 induction already reported in chapter 3.1.1 (see above). The mechanism of the significantly decreased thyroxine (T4) levels in the high dose males is not clear, however, a relationship to treatment could not be excluded.

Urine examinations revealed granular and epithelial cell casts as well as an increased number of abnormal transitional epithelial cells in urine sediments of mid and high dose males. Higher urobilinogen levels and increased number of abnormal transitional epithelial cells were found in urine specimens of high dose females. The increase in casts and transitional epithelial cells in urine sediments was considered to be a treatment-related effect and is indicative of renal dysfunction or kidney damage, respectively. The isolated finding of increased urobilinogen in the specimens of high dose females, however, is difficult to explain, since there were no concomitant changes in the clinical, hematological and pathological examinations (e.g. liver disease, hemolytic disorders), which could explain this effect. Therefore, the toxicological relevance of this finding is unclear. Moreover, increased amount of ketones were found in urine specimens of high dose animals of either sex. Due to a ketone group in the molecule, this finding is explainable by the excretion of the parent compound via the urine leading to false-positive results. The increase in calcium in serum of high dose animals was possibly also associated with renal dysfunction.

Regarding **pathology**, substance-related findings occurred in the liver, the kidneys and in the thyroid glands.

The liver weights were dose-related significantly increased in males and females of mid and high dose groups, when compared to controls. The increased liver weights correlated with a minimal to slight central hypertrophy of hepatocytes that was noted in three mid dose males and in all high dose males and females. In addition, two high dose males showed a peripheral hypertrophy of hepatocytes. The centrolobular liver cell hypertrophy associated with increased mean absolute and relative liver weights is indicative of adaptive enzyme induction with the aim to increase the metabolizing and/or excretory capacity of the liver cells. As long as no regressive cell or remarkable necrosis of liver cells occur, adaptive liver cell hypertrophy is in general reversible (Glaister, 1986).

In high dose males, the absolute and relative kidney weights were significantly increased. A minimal to moderate chronic nephropathy was observed in most of high dose males (9 out of 10), whereas only one control male showed this finding in a minimal degree. Furthermore, eosinophilic droplets were noted in tubular epithelial cells of the kidneys. Most of males in control and treatment groups showed this finding, but the amount of eosinophilic droplets was dose-related increased in mid and high dose groups. The eosinophilic droplets represent alpha2u-globuline which was

confirmed by special staining (Mallory-Heidenhain for severity) and immunohistochemical examination for specifity. The increased kidney weights in high dose males were related to the occurrence of chronic nephropathy and the higher amount of alpha2u-globuline in these animals. Under physiological conditions, alpha2u-globuline is synthesized in the liver of male rats and excreted through the kidneys. Chemicals that bind to the protein can aggravate or prevent its excretion, thus accumulating a protein-chemical-complex in the cells of the proximal tubulus and increasing the kidney weight. Normal female rats and higher species, such as humans, do not develop these changes and they are regarded as a specific phenomenon in male rats (see for review Haschek WM and Rousseaux CG, 1998). Therefore, alpha2u-globuline accumulation appears to be a unique feature of male rats and is not known to occur in other species, including man.

In high dose females, the mean relative kidney weight was significantly increased, when compared to controls. Although there were no histopathological correlates, the increased kidney weight is considered as substance-related possibly due to increased metabolic activity of the renal cells associated with metabolism and/or excretion of the compound via the urine.

In the thyroid glands, a flaky appearance of the colloid ("altered colloid") was noted in most of the males throughout all groups. In high dose males, the degree of severity of this finding was higher than in controls. In females, altered colloid was observed in a high incidence (9 out of 10) with higher gradings in the high dose group, when compared to controls. One control female showed altered colloid in the thyroid gland. It could not be excluded that the higher degree of altered colloid in high dose males as well as the high incidence with higher degrees in high dose females was related to the administration of the test substance.

Inhalative and dermal animal studies are not available.

Conclusion

The administration of β-ionone over a period of 3 months at dietary concentrations of 100, 1000 and 10 000 ppm (7 and 8 mg/kg bw/day, 72 and 83 mg/kg bw/day or 720 and 801 mg/kg bw/day for males and females) to rats lead to signs of general systemic toxicity at the high and mid dose. Target organs were liver, kidneys and thyroid glands. The liver findings in both sexes and the increased kidney weights in high dose females were indicative of adaptive and most likely reversible processes with the aim to increase the metabolizing and/or excretory capacity of these organs.

The findings in males with respect to kidneys as well as kidney relevant parameters should be seen in the light of high amounts of alpha2u-globuline in these animals. The accumulation of this protein appears to be a unique feature of male rats and is not known to occur in other species, including man. No signs of neurotoxicity were observed during functional observational battery as well as measurement of motor activity performed towards the end of the administration period. Thus, the no-observed- -effect-level (NOEL) under the conditions of the present study was 100 ppm for both sexes (about 7 and 8 mg/kg bw/d for males and females) based on adaptive liver effects in both sexes and minor urine findings in males at 1000 ppm which correspond to a dosage of 72 and 83 mg/kg bw/day for males and females (no-observed-adverse-effect-level, NOAEL).. The lowest-observed-adverse-effect-level (LOAEL) was found at 10 000 ppm (720 and 801 mg/kg bw/day for males and females) due to liver, kidney and thyroid findings in both sexes.

3.1.6 Mutagenicity

Two bacterial in vitro assays and one mammalian in vivo mutagenicity study have been located for β-ionone.

Studies in Animals

In vitro Studies

Mortelmans et al. (1986) reported results of a fully reliable Ames test with *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 with and without S-9 metabolic activation from Aroclor-1254 treated rats and hamsters with preincubation, using β-ionone (14901-07-6). The method applied is comparable to OECD TG 471 with the exception that only four instead of five *Salmonella* strains were used. Though the study has a good quality, it should be noted that due to high bacterial toxicity of the test material only low amounts could be tested. Based on a toxicity prescreen with TA100 with and without S-9 mix, 180 and 50 µg/plate, respectively were chosen as the highest test concentrations. Five dose levels with three plates per dose level were tested in the main experiment. In summary, there was no dose-related increase in the number of his^+ revertants over background in any the test strains. Neither β-ionone per se nor any of its S-9 mix metabolites were mutagenic in the Ames test with four different strains of *S. typhimurium* under the conditions of the study.

In another publication, Florin et al. (1980) screened various tobacco smoke compounds including β-ionone for mutagenicity in an assay according to Ames et al. (1975) with histidine-requiring *S. typhimurium* strains TA98, TA100, TA1535 and TA1537. Full details as to the test procedure and the preparation of the S-9 mix are in the publication. β-Ionone was not mutagenic to strains TA98, TA100, TA1535 or TA1537 in spot tests with and without metabolic activation at a concentration of 3 µmol/plate (= 576 µg/plate). While this is an older publication that states the negative results (as for β-ionone) only summarily, the detailed methods, positive controls and quality control measures support the validity of the data.

In vivo Studies

β-Ionone was tested for its ability to induce micronuclei in bone marrow erythrocytes in mice using a single intraperitoneal dose up to 750 mg/kg bw under OECD TG 474 and GLP conditions (BASF AG, 2003f). Five animals per dose level and group were used. The high and mid dose level (750 and 500 mg/kg bw) produced in all treated animals evident signs of toxicity (poor general state, irregular respiration, squatting posture) which were reversible after two days. At the low dose (250 mg/kg bw), only minor signs of clincal toxicity were observed after 2 and 4 hours of administration. The test substance did not have a chromosome-damaging (clastogenic) effect and there were no indications of any impairment of chromosome distribution in the course of mitosis (aneugenic activity) in bone marrow cells in vivo.

Conclusion

β-Ionone gave no indication of a mutagenic effect in bacteria or a clastogenic potential in an in vivo mouse micronucleus test. Therefore, there is no indication of a genotoxic potential in vivo.

3.1.7 Carcinogenicity

No valid and guideline conform long-term carcinogenicity study could be located. However, results from a non-validated short-term screening test on tumor promotion in the mouse skin have been reported (Shamberger, 1971).

In vivo Studies in Animals

Dermal

Groups of 30 freshly shaved ICR Swiss female mice, 55 - 60 days old, were initiated once with a solution of 0.125 mg 7,12-dimethylbenz[a]anthracene (DMBA) dissolved in 0.25 ml acetone

applied to their backs. No additional treatments were given for 3 weeks. Then, 0.25 ml of test material (β-ionone) at a concentration of 0.04 % (ca. 4 mg/kg bw) or a mixture of 0.006 % croton resin as tumor promotor and the test material dissolved in acetone was applied to animal skin five times weekly for 18 weeks. The animals were examined weekly and the number and distribution of local tumors were noted. The animals were shaved twice monthly and weighed at monthly intervals during the experiment. The group that received DMBA and the tumor promotor were the positive control group. The negative controls received DMBA or acetone only.

β-Ionone had no significant effect on the incidence of tumors. The positive controls showed the expected results (DMBA plus croton resin resulted in 90 % animals with tumors).

Conclusion

No valid and guideline conforming long-term carcinogenicity study could be located. However, in a non-validated screening experiment investigating the possible tumor-promoting activity in the mouse skin, β-ionone did not give any indication for such an effect when tested at a low concentration.

3.1.8 Toxicity for Reproduction

Studies in Animals

Effects on Fertility

Studies specifically designed to assess reproductive toxicity were not available for an assessment. However, in a recently well-conducted, subchronic oral toxicity study with β-ionone, the scope of examinations was extended to cover also effects on reproductive organs (BASF AG, 2004h; see also chapter 3.1.5).

In this study, the compound was administered to groups of 10 male and 10 female Wistar rats at dietary concentrations of 0, 100, 1000 and 10 000 ppm for 3 months. These concentrations corresponded to dosages of about 7 and 8 mg/kg bw/day, 72 and 83 mg/kg bw/day or 720 and 801 mg/kg bw/day for males and females. At necropsy, the reproductive organs of the males (testes, epididymides, prostate gland) and females (ovaries, uterus) were weighed and assessed by gross pathology and a histopathological examination of the testes, epididymides, prostate gland and seminal vesicles, ovaries, uterus, oviducts and vagina was subsequently performed. Furthermore, immediately after necropsy, the right testis and cauda epididymis were taken from all male animals. Sperm motility, sperm morphology and sperm head count (cauda epididymis and testis) were examined.

No treatment related changes in sperm analysis were observed. The weights of the reproductive organs were not influenced by the exposure, except a significantly increased absolute and relative weight of the testes (+ 12 % and + 18 %) and relative weight of epididymides (+ 11 %) in the high dose males (most likely as a result of the decreased mean terminal body weight in these animals). No histological abnormalities in the sex organs were detected and no histological correlates were obtained for the changes in weights of the testes and epididymides. Considering the normal biological variation of these organ weight parameters in connection with the reduced terminal body weight of the high dose animals these observations were not regarded as adverse effects.

In the females, estrous cycle was not changed compared to controls at any dose and histopathology did not reveal any treatment related effects on sex organs.

Developmental Toxicity

A few studies, which investigated the effects of β-ionone on the developing organism have been reported in the literature. However, only one of them is well documented with regard to methods and results and was conducted according to current valid guideline requirements and under GLP conditions.

In this GLP conforming study, β-ionone was tested for its prenatal developmental toxicity in Wistar rats according to OECD TG 414 (BASF AG, 2004i). The test substance was administered as a solution in olive oil to 25 time-mated female Wistar rats/group by stomach tube at doses of 25, 100 and 400 mg/kg bw on day 6 through day 19 post coitum (p.c.). A standard dose volume of 5 ml/kg bw was used for each group. The control group, consisting of 25 females, was dosed with the vehicle only. 23 - 25 females/group had implantation sites at terminal sacrifice. Food consumption and body weights of the animals were recorded regularly throughout the study period. The state of health of the animals was checked each day.

On day 20 post coitum, all females were sacrificed and assessed by gross pathology (including weight determinations of the liver, the unopened uterus and the placentae). For each dam, corpora lutea were counted and number and distribution of implantation sites (differentiated as resorptions, live and dead fetuses) were determined. The fetuses were removed from the uterus, sexed, weighed, and further investigated for any external findings. Thereafter, nearly one half of the fetuses of each litter were examined for soft tissue findings and the remaining fetuses for skeletal (incl. cartilage) findings. The following substance-related findings were obtained:

High dose group (400 mg/kg bw/day):

- transient salivation in all rats immediately after gavaging on days 7 - 19 p.c.
- discolored urine in a total of 21 out of 25 dams (days 12 - 20 p.c.), not considered a toxicological finding
- significantly reduced food consumption on days 6 - 8 p.c. (about –9 %)
- significant impairments in absolute body weight gain on days 8 - 10 p.c. (about –29 %)
- lower corrected body weight gain (about –17 %, not statistically significant)
- significantly increased absolute and relative liver weights (about +29 %)
- no substance-related effects on gestational parameters or fetuses

Mid dose group (100 mg/kg bw/day):

- transient salivation in 22 out of 25 rats immediately after gavaging between treatment days 12 - 19 p.c.
- statistically significantly increased absolute and relative liver weights (about 8 or 9 % above controls)
- no substance-related effects on gestational parameters or fetuses

Low dose group (25 mg/kg bw/day):

- no substance-related adverse effects on dams, gestational parameters or fetuses

In summary, the oral administration of β-ionone to pregnant Wistar rats from implantation to one day prior to the expected day of parturition (days 6 - 19 p.c.) elicited substance-induced effects on the dams including signs of maternal toxicity at 400 mg/kg bw/day. 100 mg/kg bw/day resulted in some substance-related findings (i.e. temporary salivation, marginally increased liver weights), which are, however, not considered to be adverse, but mirror some adaptive responses of the animals. At the low dose (25 mg/kg bw/day) no substance-induced effects on the dams occurred.

The test substance had no influence on gestational parameters and induced no adverse signs of developmental toxicity up to and including the high dose level; especially, no indications of teratogenic effects occurred which could be causally related to the test substance administration.

Conclusion

In a well-conducted 90 days study in rats according to OECD TG 408 with administration of the test substance in the diet, β-ionone does not have potential to damage the reproductive organs at least up to the highest tested concentration of 10 000 ppm (720 and 801 mg/kg bw/day for males and females).

Based on the results of a GLP and guideline-conforming developmental toxicity study with gavage application of β-ionone, the no-observed-adverse-effect-level (NOAEL) for maternal toxicity was 100 mg/kg bw/day. The NOAEL for prenatal developmental toxicity could be fixed at the highest tested dose (400 mg/kg bw/day). The test substance had no influence on gestational parameters and induced no adverse signs of developmental toxicity and in particular no indications of teratogenic effects up to and including the highest dose level were observed.

3.2 Initial Assessment for Human Health

From animal experiments it can be concluded that β-ionone is absorbed after oral exposure. Metabolism takes mainly place in the liver. Metabolites, which were identified in the urine of exposed rabbits are 3-oxo-β-ionone, 3-oxo-β-ionol, dihydro-3-oxo-β-ionol, 3-hydroxy-β-ionol and glucuronic acid conjugates. β-Ionone was found to be an inducer of CYP 1A and 2B isozymes in the liver of rodents.

β-Ionone has only low acute toxicity after oral ingestion. A gavage study conducted with a mixture of 60 % α-ionone and 40 % β-ionone revealed a LD_{50} of 4590 mg/kg bw. Clinical signs of toxicity were depression and tremors.

In studies conducted according to OECD test guidelines and under GLP conditions, β-ionone was not irritating to the skin of rabbits after semiocclusive application for 4 hours and only slightly irritating to the eyes. After a 24-hours exposure under occlusive conditions, a slight irritation of the skin was observed in rabbits. A limited human patch test did not reveal a potential for skin irritation when a not further specified mixture of α- and β-ionone was applied undiluted to the skin of volunteers.

A limited Guinea pig maximisation test found no evidence that β-ionone is a dermal sensitiser. According to secondary sources, ionone (a not further specified mixture of α- and β-ionone) was negative in an Open Epicutaneous Test with Guinea pigs as well as in a human maximisation test with a product containing 97.5 % α-ionone and 2.5 % β-ionone.

The administration of β-ionone over a period of 90 days according to OECD TG 408 at dietary concentrations of 100, 1000 and 10 000 ppm (7 and 8 mg/kg bw/day, 72 and 83 mg/kg bw/day or 720 and 801 mg/kg bw/day for males and females) to rats led to signs of general systemic toxicity at the high and mid dose. Target organs were liver, kidneys and thyroid glands. The liver findings in both sexes and the increased kidney weights in high dose females were indicative of adaptive and most likely reversible processes with the aim to increase the metabolizing and/or excretory capacity of these organs. The findings in males with respect to kidneys as well as kidney relevant parameters should be seen in the light of high amounts of alpha2u-globuline in these animals. The occurrence of alpha2u-globuline was confirmed by immunohistochemical examination. The accumulation of this protein appears to be a unique feature of male rats and is not known to occur in other species, including man. No signs of neurotoxicity were observed during functional observational battery as well as measurement of motor activity performed towards the end of the administration period.

Thus, the no-observed-effect-level (NOEL) under the conditions of the present study was 100 ppm for both sexes (about 7 and 8 mg/kg bw/day for males and females) based on adaptive liver effects in both sexes and minor urine findings in males at 1000 ppm which correspond to a dosage of 72 and 83 mg/kg bw/day for males and females (no-observed-adverse-effect-level, NOAEL). The lowest-observed-adverse-effect-level (LOAEL) was found at 10 000 ppm (720 and 801 mg/kg bw/day for males and females) due to liver, kidney and thyroid findings in both sexes.

β-Ionone gave no indication of a mutagenic effect in bacteria or a clastogenic potential in an in vivo mouse micronucleus test. Therefore, there is no indication of a genotoxic potential in vivo.

No studies that would be considered adequate for the evaluation of carcinogenic potential were available. A short-term screening experiment investigating a tumor-promoting potential on mouse skin did not indicate such an effect at a low test concentration.

In a well-conducted 90 days study in rats according to OECD TG 408 with administration of the test substance in the diet, β-ionone did not have the potential to damage the reproductive organs up to the highest tested concentration of 10 000 ppm (720 and 801 mg/kg bw/day for males and females).

Based on the results of a GLP and guideline conforming developmental toxicity study (OECD TG 414) with gavage application of β-ionone, the no-observed-adverse-effect-level (NOAEL) for maternal toxicity was 100 mg/kg bw/day. The NOAEL for prenatal developmental toxicity could be fixed at the highest tested dose (400 mg/kg bw/day). The test substance had no influence on gestational parameters and induced no adverse signs of developmental toxicity and in particular no indications of teratogenic effects up to and including the highest dose level were observed.

4 HAZARDS TO THE ENVIRONMENT

4.1 Aquatic Effects

The results from aquatic toxicity tests available were summarized below to evaluate the toxicity of β-ionone to aquatic organisms.

Acute Toxicity Test Results

Fish:

Acute toxicity to the fathead minnow, *Pimephales promelas*, was determined under flow-through conditions following the US EPA Committee on Methods for Toxicity Tests with Aquatic Organisms (1975). Fish were exposed to five test concentrations ranging from 1.47 to 8.22 mg/l (nominal) and 1.42 to 6.98 mg/l (measured) and control water. The LC_{50} (96 h) was 5.1 mg/l. Behavioral effects (loss of schooling behavior, hypo activity and under reactivity to external stimuli) were observed in 10 % of the fish exposed to 3.47 mg/l (nominal) after 24, 48 and 72 hours, but not after 96 hours and in 15 - 20 % of the fish exposed to 5.34 mg/l (nominal). All fish exposed to a nominal concentration of 8.22 mg/l had died after 24 hours of exposure (Geiger, Call and Brooke, 1988).

This value is in good correspondence with a a LC_{50} (96 h) for *Leuciscus idus* with 6.8 mg/l (BASF AG, 1989d) and a LC_{50} (48 h) for the rainbow trout *Oncorhynchus mykiss* between 5 – 10 mg/l (Hoffmann LaRoche Inc., 1989), and with, even if the latter study only has a reliability rating of 4.

Invertebrates:

Two EC_{50} - values are available for the water flea *Daphnia magna*:

Acute toxicity (48 h) to the water flea *Daphnia magna* was determined according to the OECD Guideline 202, under GLP conditions. The test substance was tested in the range of concentrations between 1 and 16 mg/l using a static system. The dilution factor was 2. Concentration control analyses performed by HPLC indicate that the initial concentration was between 90 and 92 % of nominal concentration and reduction during the test was just below 20 %. The EC_{50} (48 h) for the endpoint immobilization was 3.7 mg/l based on initial measured concentrations (BASF AG, 2004j).

In a static test according to DIN 38412 (part 11) an EC_{50} (48 h) of 1.1 mg/l could be determined: This value is based on nominal concentrations, because no concentration control analysis were performed (BASF AG, 1990).

Due to the higher reliability the result of the first study is used for the assessment of β-ionone.

Algae:

Acute toxicity of β-ionone to the green alga *Scenedesmus subspicatus* was determined according to the German Industrial Standard Guideline DIN 38412, part 9, using five test concentrations ranging from 2.5 to 50 mg/l (nominal), a control and a solvent control (5 mg/l of Cremophor). The $E\mu C_{50}$ (72 h) for the endpoint growth rate was 22.2 mg/l (nominal), the EbC_{50} (72 h) for the endpoint biomass was 21.2 mg/l (nominal) (BASF AG, 1989e). The EC_{10} (72 h) for growth rate and biomass were 7.1 mg/l and 6.2 mg/l, respectively.

Chronic Toxicity Test Results

No data on chronic toxicity of β-ionone to aquatic organisms are available.

Toxicity to Microorganisms

In a respiration inhibition test according to ISO 8192, the effect of β-ionone on the respiration rate of activated sludge from a municipal sewage treatment plant was investigated using three test concentrations ranging from 60 to 1000 mg/l (nominal). The EC_{20} (0.5 h) was approximately 53 mg/l, the EC_{50} (0.5 h) was approximately 1000 mg/l (BASF AG, 1988a). In a further respiration inhibition study (OECD 209) using activated sludge, th effects of 4 concentrations (25-500 mg/l) was examined during a 3-hour exposure at 20 °C. The EC50 was determined to lie in the range 100 – 120 mg/l (Givaudan, 1991).

The toxicity of β-ionone to the bacterium *Pseudomonas putida* was determined in an acute oxygen consumption test according to Robra using four test concentrations ranging from 1250 to 10,000 mg/l (nominal). The EC_{10} (0.5 h) was 8300 mg/l (BASF AG, 1988b).

4.2 Terrestrial Effects

Acute Toxicity Test Results

No data on acute toxicity of β-ionone to terrestrial organisms are available.

Chronic Toxicity Test Results

No data on chronic toxicity of β-ionone to terrestrial organisms are available.

4.3 Other Environmental Effects

The acute oral toxicity for the red-winged blackbird, *Agelaius phoeniceus*, was investigated (Schafer, Bowles and Hurlbut, 1983). Over a test period of 18 hours a LD_{50} of > 562 mg/kg bw

based on food consumption data could be derived. No information about dosage or application procedure was available.

4.4 Initial Assessment for the Environment

The colorless to yellowish liquid β-ionone has a water solubility of about 0.169 g/l and a vapor pressure of about 0.009 hPa at 25 °C. The measured log K_{OW} of 4.0, the calculated log K_{OC} of 2.80-3.34 and the calculated BCF of 501 indicate a potential for bio- and geoaccumulation. According to distribution modeling using Mackay Level I, water (34 %), soil (27 %), sediment (27 %) and air (12%) are the main targets for the compound. β-Ionone is readily biodegradable according to OECD criteria (> 70 % degradation within 28 days, 10-day-window criteria fulfilled). In the atmosphere, based on a 24-hour day, it will be rapidly photodegraded by reactions with OH radicals (calculated $t_{1/2}$: 1.6 h) and ozone (calculated $t_{1/2}$: 18 min).

Results of most valid studies on acute aquatic toxicity are available for fish (*Pimephales promelas*; LC_{50} (96 h): 5.1 mg/l), invertebrates (*Daphnia magna*; EC_{50} (48 h): 3.7 mg/l) and algae (*Scenedesmus subspicatus*; $E\mu C_{50}$ (72 h) 22.2 mg/l, EbC_{50} (72 h) 21.2 mg/l). Based on these acute toxicity studies, β-ionone is considered as toxic to aquatic organisms. It may cause long-term adverse effects in the aquatic environment. No results on prolonged or chronic toxicity to aquatic organisms are available. According to the EU risk assessment procedure, a $PNEC_{aqua}$ of 3.7 µg/l was obtained by applying an assessment factor of 1000 on the lowest $L(E)C_{50}$, the result of the test using the invertebrate *Daphnia magna*.

β-Ionone was found in concentrations up to 1.2 µg/l in waters of lakes and rivers mainly as a result of biotransformation processes in phytoplankton species. If compared with the $PNEC_{aqua}$ of 3.7 µg/l it can be concluded that the measured levels are limited in coverage and they may therefore include β-ionone from non-anthropogenic sources.

5 RECOMMENDATIONS

Human Health: The only hazards identified are slight irritation to the eyes and changes in the liver, kidneys and thyroid after repeated oral exposure that were either of minor severity or were considered to be a species-specific effect in male rats. Given the main use as a chemical intermediate and the low content of the substance in consumer products in the Sponsor country, the substance is considered to be of low priority for further work. Countries may desire to investigate any exposure scenarios that were not presented by the Sponsor country.
The chemical is currently of low priority for further work.

Environment: The chemical possesses properties indicating a hazard for the environment. Based on the data presented by the Sponsor country (relating to production by one producer which accounts for approx. 10 – 40 % of global production and relating to the use in several OECD countries), exposure to the environment from human production and use of beta-ionone is anticipated to be low, and therefore this chemical is currently of low priority for further work. Countries may desire to investigate any exposure scenarios that were not presented by the Sponsor country.
The chemical is currently of low priority for further work.

6 REFERENCES

Angelopoulou D, Demetzos C, Dimas C, Perdetzoglou D and Loukis A (2001). Essential oils and hexane extracts from leaves and fruits of *Cistus monspeliensis* – cytotoxic activity of ent-13-epi-manoyl oxide and its isomers. Planta Med. **67**, 168-171.

Anonymous (2001). Indian roses smell different. COSSMA **3**, 44-45.

Ames BN, McCann J and Yamasaki EC (1975). Methods for detecting carcinogens and mutagens with Salmonella / mammalian microsome mutagenicity test. Mut. Res. **31**, 347-364.

Baglay AK, Gurarly LL and Kuleshov GG (1988). Physical properties of compounds used in vitamin synthesis. J. Chem. Eng. Data **33**, 512-518.

BASF AG (1988a). Laboratory of Ecology. Pruefbericht ueber eine Untersuchung der Wirkung von beta-Ionon auf die Atmungsaktivitaet von Belebtschlamm. Unpublished report. Project No. 01.0429. 1988.

BASF AG (1988b). Laboratory of Ecology. Oxygen consumption according to Robra. Unpublished report. Project No. 0909/88. 11 Aug 1988.

BASF AG (1989a). Analytical Laboratory. Dampfdruck: 4-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-3-buten-2-on). Unpublished report. Project No. BRU 89.178. 6 Jul 1989.

BASF AG (1989b). Analytical Laboratory. Bestimmung des Verteilungskoeffizienten Log Pow der Prüfsubstanz in 1-Octanol/Wasser bei 25°C. Unpublished report. Project No. 106288/11. 2 May 1989.

BASF AG (1989c). Laboratory of Ecology, Pruefbericht ueber eine Untersuchung auf biologische Abbaubarkeit im respirometrischen Test (EG-Methode). Unpublished report. Project No. 388429. 2 Mar 1989.

BASF AG (1989d). Department of Toxicology. Golden Orfe (*Leuciscus idus* L., golden variety) - Report on the study of the acute toxicity. Unpublished report. Project No. 10F0606/885329. 10 Jun 1989.

BASF AG (1989e). Laboratory of Ecology. Untersuchungsbericht Prüfung auf Ökotoxizität: Hemmung der Algen-Zellvermehrung nach DIN 38412 L9. Unpublished report. Project No. 01/89/1485. 6 Dec 1989.

BASF AG (1990) Department of Product Safety. Laboratory of Ecology. Akute Toxizität für Daphnien nach DIN 38412 L11. Unpublished study. 01/89/1485, 15 Jan 1990.

BASF AG (1992a). Department of Toxicology. Report on the acute dermal irritation / corrosivity to the intact dorsal skin of beta-ionone in white rabbits. Unpublished report. Project No. 18H0245/912113. 13 May 1992.

BASF AG (1992b). Department of Toxicology. Report on the acute irritation to the eye of beta-ionone in white rabbits. Unpublished report. Project No. 18H0245/912114. 13 May 1992.

BASF AG (2003a). Technical Data Sheet beta-Ionon R. ME 018 d/e. 08/2003.

BASF AG (2003b). Product Safety. Data assessment. Unpublished calculation. SRC HENRYWIN v3.10, 4 Dec 2003.

BASF AG (2003c). Product Safety. Data assessment. Unpublished calculation. SRC AOP v1.90. 4 Dec 2003.

BASF AG (2003d). Product Safety. Data assessment. Unpublished calculation. SRC AOP v1.90. 9 Dec 2003.

BASF AG (2003e). Product Safety. Data assessment. Unpublished calculation. SRC PCKOCWIN v1.66. 4 Dec 2003.

BASF AG (2003f). Product Safety. Cytogenetic study in vivo with beta-Ionon R in the mouse micronucleus test, single intraperitoneal administration. Unpublished report. Project No. 26M0449/024134. 02/0449-1. 9 Dec 2003.

BASF AG (2004a) Analytical report. Unpublished data. H-NMR spectra of beta-Ionone (BASF AG R Ch81721616K0). 26 Aug 2004.

BASF AG (2004b) Analytical report. Unpublished data. H-NMR spectra of beta-Ionone (beta-Ionen DSM). 26 Aug 2004.

BASF AG (2004c). Safety data sheet – beta-Ionon. 27 Aug 2004.

BASF AG (2004d). Internal data. pers. Communications. Nov 2004.

BASF AG (2004e). Unpublished data (Emission of beta-Ionon cited in the German Emission Register 2000). 11 May 2004.

BASF AG (2004f). Department of Product Safety. Expert Judgment on Hydrolysis. 10 Nov 2004.

BASF AG (2004g). Product Safety. Data assessment. Unpublished calculation. Mackay Level I V2.11. 09 Nov. 2004.

BASF AG (2004h). Product Safety. beta-Ionon R - Subchronic toxicity study in Wistar rats, administration in the diet for 3 months. Unpublished report. Project No. 50S0449/02057. 02/449-1. 4 Jun 2004.

BASF AG (2004i). Product Safety. beta-Ionon R - Prenatal developmental toxicity study in Wistar rats, oral administration (gavage). Unpublished report. Project No. 30R0449/02056. 02/449-1. 19 May 2004.

BASF AG (2004j). Product Safety. beta-Ionon R - Determination of the acute effect on the swimming ability of the water flea *Daphnia magna* STRAUS. Unpublished report. Project No. 50E0449/023002. 19 Aug 2004.

BASF AG (2005). Product Safety. Data assessment. Unpublished calculation. SRC MPBPWIN 1.41. 15 Aug 2005.

Beilstein, Handbook (online), BRN 1909545, BPR 79-77-6, Pfizer & Co. US 2638483, 1951, Handbook

CFIA (2004). Canadian Food Inspection Agency, http://www.inspection.gc.ca/english/anima/feebet/sched5/schvisie.shtml.

Chorus I, Klein G, Fastner I and Rotard W (1992). Off-flavours in surface waters - how efficient is bank filtration for their abatement in drinking water? Water Sci. Technol. **25**, 251-258.

Cotsaris E, Bruchet A, Mallevialle J and Bursill DB (1995). The identification of odorous metabolites produced from algal monocultures. Water Sci. Technol. **31**, 251-258.

EU (1999). Regulation (EC) No 2232/96 of the European Parliament and of the Council of 28 October 1996. Community procedure for flavouring substances used or intended for use in or on foodstuffs. 23 Nov 1999.

Evans WG (1994). Volatile organic chemicals of a shore-dwelling cyanobacterial mat community. J. Chem. Ecol. **20**, 219-230.

Ferreira V, Ardanuy M, López R and Cacho JF (1998). Relationship between flavor dilution values and odor unit values in hydroalcoholic solutions: role of volatility and a practical rule for its estimation. J. Food Chem. **46**, 4341-4346.

FFCR Japan (2004). The Japan Food Chemical Research Foundation, http://www.ffcr.or.jp/zaidan/FFCRHOME.nsf/pages/eng.h-page.

Fichan I, Larroche C and Gros JB (1999). Water solubility, vapor pressure, and activity coefficients of terpenes and terpenoids. J. Chem. Eng. Data **44**, 56-62.

Flamini G, Cioni PL, Morelli I, Ceccarini L, Andolfi L and Macchia M (2003). Composition of the essential oil of *Medicago marina* L. from the coastal dunes of Tuscany, Italy. Flav. Fragr. J. **18**, 460-462.

Florin I, Rutberg L, Curvall M an Enzell CR (1980). Screening of tobacco smoke constituents for mutagenicity using the Ames' test. Toxicology. **18**, 219-232.

Geiger DL, Call DJ and Brooke LT (1988). Acute toxicities of organic chemicals to fathead minnows (*Pimephales promelas)*. Volume 4. Center for Lake Superior Environmental Studies, University of Wisconsin.

Givaudan (1991). Group Safety & Environmental Protection. Ionone beta synthetic. "Activated Sludge Respiration. Inhibition Test". Unpublished study. 19 Feb 1991.

Glaister JR (1986). Principles of Toxicological Pathology. Taylor & Francis, London and Philadelphia, pp 83-85.

Greif N (1967). Cutaneous safety of fragrance material as measured by the Maximization test. American Perfumer and Cosmetics. **82**, 54-57.

Gu HK, Jeong TC, Chun YJ, Yun CH, Roh JK, and Choi IK (1997). Induction of cytochrome P450 by ionones in liver lobes of Sprague Dawley rats. Korean J. Toxicol. **13(**4), 385-391.

Haschek WM and Rousseaux CG (1998). Fundamentals of Toxicologic Pathology., Academic Press, San Diego, USA, 173-176.

Hoffmann-LaRoche Inc. (1967). Acute toxicity, eye and skin irritation tests on aromatic compounds. Unpublished results, 16 Nov 1967.

Hoffmann LaRoche Inc. (1975). Prüfung im 5 Tage Toxizitätsversuch an Ratten. Unpublished results, 10 Nov 1975.

Hoffmann LaRoche Inc. (1989). Data compilation, ecotoxicological evaluation, beta-ionone. Unpublished data, E-29/89, 02 Aug 1989.

Ide H and Toki S (1970). Metabolism of β-ionone. Isolation, characterization and identification of the metabolites in the urine of rabbits. Biochem. J. **119**, 281-287.

JECFA (2004). http://jecfa.ilsi.org/evaluation.cfm?chemical=beta-IONONE&keyword=BETA-IONONE.

Jenner PM, Hagan EC, Taylor JM, Cook EL, and Fitzhugh OG (1964). Food flavourings and compounds of related structure I. Acute oral toxicity. Food Cosmet. Toxicology. **2**, 327-343.

Jeong TC, Kim HJ, Yun CH, Lee SS, Yang KH, Han SS and Roh JK (1995). Induction of liver cytochrome P450 2B1 by beta-ionone in Sprague Dawley rats, Biochem. Biophy. Res. Commun. **216**, 198-202.

Jeong TC, Gu HK, Chun YJ, Yun CH, Han SS and Roh JK (1998). Effects of beta-ionone on the expression of cytochrome P450s and NADPH-cytochrome P450 reductase in Sprague-Dawley rats. Chemico-Biological Interactions. **114**, 97-107.

Jeong TC, HK Gu, Park J-I, Yun H-I, Kim H-C, Ha C-S and Roh JK (1999). Pretreatment of male BALB/c mice with beta-ionone potentiates thioacetamide-induced hepatotoxicity. Toxicol. Letters. **105**, 39-46.

Jeong HG, Chun Y-J, Yun C-H, Moon C-K, Lee H-S, Han SS, Lee E-S, and Jeong TC (2002). Induction of cytochrome P450 1A and 2B by alpha- and beta-ionone in Sprague Dawley rats. Arch. Pharm. Res. **25**, 197-201.

Jones GJ and Korth W (1995). In situ production of volatile odour compounds by river and reservoir phytoplankton populations in Australia. Water Sci. Technol. **31**, 145-151.

Juettner F (1984). Dynamics of the volatile organic substances associated with cyanobacteria and algae in a eutrophic shallow lake. Appl. Environ. Microbiol. **47**, 814-820.

Juettner F (1992). Flavour compounds in weakly polluted rivers as a means to differentiate pollution sources. Water Sci. Technol. **25**, 155-164.

Katz A (1946). Spice Mill **69**. pp 46. **In:** Opdyke DLJ (1979). Monographs on fragrance raw materials. Ionone, 444-445.

Klecak G (1985). The Freund's Complete Adjuvant Test and the Open Epicutaneous Test. Curr. Probl. Dermatol. **14**, 152-171.

Korth W, Ellis J and Bowmer K (1992). The stability of geosmin and MIB and their deuterated analogues in surface waters and organic solvents. Water Sci. Technol. **25**, 115-122.

Lazari DM, Skaltsa HD and Constantinidis T (2000). Volatile constituents of *Cerastium candidissimum*, a Greek endemic species. Flav. Fragr. J. **15**, 174-176.

MacTavish HS and Menary RC (1997). Volatiles in different floral organs, and effect of floral characteristics on yield of extract from *Boronia megastigma* (Nees). Ann. Botany **80**, 305-311.

MacTavish H and Menary RC (2000a). Production of volatiles in brown boronia flowers after harvest: pilot scale research. J. Horticult. Sci. & Biotechnol. **75**, 455-458.

MacTavish H, Davies NW and Menary RC (2000b). Emission of volatiles from brown boronia flowers: some comparative observations. Ann. Botany **86**, 347-354.

Mendelsohn HV (1946). Lemon grass oil. Archs. Derm. Spyh. **53**, 94-98.

Moehren S and Juettner F (1983). Odorous compounds of different strains of Anabaena and Nostoc (Cyanobacteria). Water Sci. Technol. **15**, 221-228.

Mortelmans K, Haworth S, Lawlor T, Speck W, Tainer B and Zeiger E (1986). Salmonella Mutagenicity Tests: II. Results from the testing of 270 chemicals. Environmental Mutagenesis **8**, **Suppl. 7**, 1-119.

Ômura H, Honda K and Hayashi N (2000). Floral scent of *Osmanthus fragrans* discourages foraging behavior of cabbage butterfly, *Pieris rapae*. J. Chem. Ecol. **26**, 655-666.

Opdyke DLJ (1979). Monographs on fragrance raw materials. Pergamon Press. Ionone, pp 444.

Plummer JA, Wann JM, Orifici R and Spadek ZE (1998). Effects of photon flux density on photosynthesis, growth, flowering, and oil content in Boronia. Aust. J. Agric. Res. **49**, 791-797.

Plummer JA, Wann JM and Spadek ZE (1999). Intraspecific variation in oil components of *Boronia megastima* Nees (Rutaceae) flowers. Ann. Botany **83**, 253-262.

Prelog V and Meier HL (1950). Über die biochemische Oxydation von β-Ionon im Tierkörper. Helv. Chim. Acta **33**, 1276-1285.

Puri HS, Siddiqui MS, Akhila A and Naqvi A (1985). Essential oil of *Thymus serpyllum*: a source of thymol in India. Parfümerie und Kosmetik **66**, 606-609.

RIFM (2000). Research Institute on Fragrance Materials Inc., Ionone Beta: Determination of ready biodegradability (CO2-Evolution test). Unpublished study, No. BL6803/B, finalised at 3rd May 2000.

Roempp (2003). Roempp Online, ©. Georg Thieme Verlag, status 18 March 2004.

Roberts NJ and Menary RC (1994). Effect of nitrogen on growth, flower yield, oil composition, and yield in *Boronia megastima* Nees. J. Plant Nutrit. **17**, 2035-2052.

Sabljic A and Guesten H (1995). QSARs for soil sorption. **In**: Overview of structure-activity relationships for environmental endpoints. Edited by JLM Hermens. Report, EU-project EV5V-CT92-0211.

Salt SD, Tuzun S and Kuc J (1986). Effects of ß-ionone and abscisic acid on the growth of tobacco and resistance to blue mold. Micry of effects of stem infection by Peronospora tabacina Adam. Physiol. Molecul. Plant Pathol. **28**, 287-297.

Schafer EW, Bowles WA Jr. and Hurlbut J (1983). The acute oral toxicity, repellency, and hazard potential of 998 chemicals to one or more species of wild and domestic birds. Arch. Environ. Contam. Toxicol. **12**, 355-382.

Shahidi F, Rubin LJ and D`Souza LA (1986). Meat flavor volatiles: a review of the composition, techniques of analysis and sensory evaluation. CRC Crit. Rev. Food Sci. Nutrition **24**, 141-243.

Shamberger RJ (1971). Inhibitory effect of vitamin A on cacinogenesis. J. Nat. Cancer Inst. **47**, 667-673.

SPIN (2004). SPIN database http://www.spin2000.net, query November 2004.

Swiss Product Register (2004). pers. communication, P. Bormann, query November 2004.

Takasago International Corporation (1999). Beta-Ionone (CAS-no.: 14901-07-6). Toxicology studies in the Guinea pig. Final report. Acute dermal irritation / photoxicity study, skin sensitisation: Magnusson and Kligman Maximisation. Unpublished report. Test No. P-4908, 4 Aug 1999.

Tellez M, Khan IA, Kobaisy M, Schrader KK, Dayan FE and Osbrink W (2002). Composition of the essential oil of *Lepidium meyenii* (Walp.). Phytochemistry **61**, 149-155.

TGD (2003). Technical Guidance Document on Risk Assessment in support of Commission Directive 93/67/EEC on risk assessment of new notified substances, Commission Regulation (EC) No. 1488/94 on risk assessment for existing substances and Directive 98/8/EC of the European

Parliament and of the Council concerning the placing of biocidal products on the market. Part III. European Commission Joint Research Centre. EUR 20418 EN/3.

Thomas RG (1982). Volatilization from water. **In:** Handbook of chemical property estimation methods, environmental behavior of organic compounds. Edited by WJ Lyman, WF Reel, and DH Rosenplatt. McGraw-Hill Book Company, 15.1-15.34.

Ullmann's Encyclopedia (2000). Sixth Edition. 2000 Electronic Release. 2000 Wiley-VCH Verlag GmbH. Weinheim, Germany.

Veith GD, Defoe DL and Bergstedt BV (1979). Measuring and estimating the bioconcentration factor of chemicals in fish. J. Fish Board Can. **36**, 1040-1948.

Walsh K, Jones GJ and Dunstan RH (1998). Effect of high irradiance and iron on volatile odour compounds in the cyanobacterium *Microcystes aeruginosa*. Phytochemistry **49**, 1227-1239.

Wilkesmann R, Schlett C and Their HP (1995). Determination of ultra traces of odors in water after solid phase extraction and closed-loop stripping analysis (CLSA). Vom Wasser **84**, 357-367.

Zeringue Jr HJ (1997). Volatile antifungal compounds in maize kernels: effects of ear position on aflatoxin production, J. AOAC Internat. **80**, 341-344.

S I D S

D o s s i e r

Existing Chemical	ID: 79-77-6
CAS No.	79-77-6
EINECS Name	(E)-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-3-buten-2-one
EC No.	201-224-3
Molecular Weight	192.30 g/mol
Molecular Formula	C13 H20 O

Producer Related Part

Company:	BASF AG
Creation date:	18-FEB-1992

Substance Related Part

Company:	BASF AG
Creation date:	18-FEB-1992

Status:	other: The consortium wants to state that this document is declared confidential within the framework of the ICCA/HPV-Prog. and not ready for publication via OECD/WHO. This has to be derestricted by BASF before publication is allowed.
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Flags (profile):	Flags: without flag, SIDS

1.0.1 Applicant and Company Information

Type: lead organisation
Name: BASF AG
Contact Person: Dr. Rolf Sarafin **Date:**
, GUP/CL - Z570
Street: Carl-Bosch-Strasse
Town: 67056 Ludwigshafen
Country: Germany
Phone: +49 621 60 44712
Telefax: +49 621 60 58043
Email: rolf.sarafin@basf.com
Homepage: www.basf.com

Flag: Critical study for SIDS endpoint
03-JAN-2006

Type: cooperating company
Name: DSM Nutritional Products AG
Contact Person: Dr. Michael Matthes **Date:**
SHE Manager
Bau 241 / Raum 643
Street: PO Box 3255
Town: 4002 Basel
Country: Switzerland

Flag: Critical study for SIDS endpoint
06-OCT-2005

1.0.2 Location of Production Site, Importer or Formulator

1.0.3 Identity of Recipients

1.0.4 Details on Category/Template

1.1.0 Substance Identification

IUPAC Name: 4-(2,6,6-trimethylcyclohex-1-ene-1-yl)-but-3-ene-2-one
Mol. Formula: C13 H20 O
Mol. Weight: 192.30 g/mol

Flag: Critical study for SIDS endpoint
16-AUG-2005

1.1.1 General Substance Information

Purity: 97 - % w/w
Odour: dry powdery, floral, woody, orris

Remark: taste: woody, fruity, floral, dry
-
Test substance: beta-Ionon R (BASF)
Flag: Critical study for SIDS endpoint

13-AUG-2004 (1)

Physical status: liquid
Purity: >= 97 - % w/w
Colour: colourless to pale yellowish
Odour: woody, dry, floral

Test substance: beta-Ionon R (BASF)
Flag: Critical study for SIDS endpoint
18-MAR-2004 (2)

Physical status: liquid
Colour: clear, colourless to pale yellowish
Odour: woody, dry, floral, fruity

Test substance: beta-Ionon R (BASF)
Flag: Critical study for SIDS endpoint
13-AUG-2004 (3)

Physical status: liquid
Colour: colourless
Odour: woody, violet-like

Remark: odour threshold: 0.007 ppb
Flag: Critical study for SIDS endpoint
15-AUG-2005 (4)

Physical status: liquid
Colour: yellowish

Remark: form: slightly viscious
-
Generally, ionones have a trans configuration.
Trans-beta-ionone rearranges to the retro compound by exposure to ultraviolet light.
-
Flag: Critical study for SIDS endpoint
19-MAR-2004 (5)

Colour: light yellow to colorless
Odour: violet odor

Flag: Critical study for SIDS endpoint
19-MAR-2004 (6)

1.1.2 Spectra

Type of spectra: other: H-NMR

Remark: Sample identification: beta-Ionon of BASF AG, R Ch81721616K0
As per analytical report, this assay of beta-Ionon is clearly 3E-configured (trans-isomer CAS-No. 79-77-6).
Flag: Critical study for SIDS endpoint
10-NOV-2004 (7)

Type of spectra: other: H-NMR

Remark: Sample identification: beta-Ionon synth. formerly Givaudan, E 500/99

As per analytical report, this assay of beta-Ionon is clearly 3E-configured (trans-isomer CAS-No. 79-77-6).
Flag: Critical study for SIDS endpoint
15-NOV-2004 (8)

Type of spectra: other: H-NMR

Remark: Sample identification: beta-Ionon of DSM
As per analytical report, this assay of beta-Ionon is clearly 3E-configured (trans-isomer CAS-No. 79-77-6).
Flag: Critical study for SIDS endpoint
15-NOV-2004 (9)

1.2 Synonyms and Tradenames

(E)-beta-Ionone

Flag: Critical study for SIDS endpoint
19-MAR-2004 (10)

3-buten-2-one, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-, (e)-

Flag: Critical study for SIDS endpoint
19-MAR-2004 (10)

3-Buten-2-one, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-, (E)- (8CI, 9CI)

Flag: Critical study for SIDS endpoint
19-FEB-1992

4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-3-buten-2-one

Flag: Critical study for SIDS endpoint
19-MAR-2004 (10)

4-(2,6,6-trimethyl-1-cyclohexenyl)-3-buten-2-one

Flag: Critical study for SIDS endpoint
19-MAR-2004 (10)

4-(2,6,6-trimethyl-1-cyclohexenyl)-3-butenone-2

Flag: Critical study for SIDS endpoint
18-MAR-2004 (1)

b-cyclocitrylideneacetone

Flag: Critical study for SIDS endpoint
19-MAR-2004 (10)

beta-Ionone

Flag: Critical study for SIDS endpoint
19-MAR-2004 (10)

fema no. 2595

Flag: Critical study for SIDS endpoint
19-MAR-2004 (10)

Ionantheme beta

Flag: Critical study for SIDS endpoint
18-MAR-2004 (1)

irisone

Flag: Critical study for SIDS endpoint
19-MAR-2004 (10)

Irisone beta

Flag: Critical study for SIDS endpoint
18-MAR-2004 (1)

trans-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-3-buten-2-one

Flag: Critical study for SIDS endpoint
19-MAR-2004 (10)

trans-beta-Ionone

Flag: Critical study for SIDS endpoint
13-AUG-2004 (1) (10)

1.3 Impurities

CAS-No: 127-41-3
EC-No: 204-841-6
EINECS-Name: 4-(2,6,6-trimethylcyclohex-2-ene-1-yl)-but-3-ene-2-one
Contents: <= 3 - % w/w

Remark: Impurities comprise mainly other beta-ionone isomers like alpha-ionone (CAS-No. 127-41-3), sum according to the specifications <=3% (w/w).
Flag: Critical study for SIDS endpoint
25-AUG-2004 (11)

1.4 Additives

EINECS-Name: RM

Remark: Beta-ionone contains no additives.
Flag: Critical study for SIDS endpoint
25-AUG-2004 (11)

1.5 Total Quantity

Remark: Production volumes for the year 2003:

World: 4,000 - 8,000 t
Europe (incl. Germany): 1,000 - 5,000 t
USA: < 1,000 t
Asia: < 2,000 t

Flag: Critical study for SIDS endpoint

15-AUG-2005 (12)

1.6.1 Labelling

Labelling: provisionally by manufacturer/importer
Symbols: (N) dangerous for the environment
Specific limits: no
R-Phrases: (51/53) Toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment
S-Phrases: (61) Avoid release to the environment. Refer to special instructions/Safety data sets

Flag: Critical study for SIDS endpoint
13-AUG-2004 (13)

1.6.2 Classification

Classified: provisionally by manufacturer/importer
Class of danger: dangerous for the environment
R-Phrases: (51/53) Toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment
Specific limits: no

Flag: Critical study for SIDS endpoint
13-AUG-2004 (13)

1.6.3 Packaging

1.7 Use Pattern

Type: industrial
Category: Chemical industry: used in synthesis

Remark: Synthetic ionones are used in the synthesis of vitamine A, damascenones and iso-methylionones.
-
Flag: Critical study for SIDS endpoint
10-NOV-2004 (4)

Type: industrial
Category: Chemical industry: used in synthesis

Remark: The key building block of all industrial vitamin A syntheses is beta-ionone ...
-
Flag: Critical study for SIDS endpoint
19-MAR-2004 (5)

Type: industrial
Category: Chemical industry: used in synthesis

Remark: vitamin A production
-
Flag: Critical study for SIDS endpoint

19-MAR-2004 (6)

Type: industrial
Category: Chemical industry: used in synthesis

Remark: Key intermediate in the synthesis of vitamin A.
-
Flag: Critical study for SIDS endpoint
19-MAR-2004 (14)

Type: industrial
Category: Personal and domestic use

21-SEP-1994

Type: use
Category: Cosmetics

Remark: Application: Fragrances
Very useful for cosmetics and especially for lipsticks.
-
Flag: Critical study for SIDS endpoint
10-NOV-2004 (3)

Type: use
Category: Cosmetics

Remark: Additives: Substances which are added to cosmetic products, often in relatively small amounts, to impart or improve desirable properties or suppress (or minimize) undesirable properties.
Perfumes: Substances which are added to cosmetic products for the purpose of perfume. This includes substances used in fragrances for their properties in mixtures (solvents, excipients, etc.).
Source: EU. Commission Decision 96/335/EC establishing an inventory and a common nomenclature of ingredients employed in cosmetic products. O.J. (L 132) 1, 1 Jun 1996.
From Part II: Perfume and Aromatic Raw Materials.
09-DEC-2003 (15)

Type: use
Category: Cosmetics

Remark: Synthetic ionones are used, under different brand names, for the production of soaps, perfumes, violet scents in general.
-
Flag: Critical study for SIDS endpoint
10-NOV-2004 (4)

Type: use
Category: Food/foodstuff additives

Remark: It is ... used in flavour compositions, such as raspberry, plum, cherry, grape, pineapple ...

Use level in the finish product: 1 to 15 ppm (up to 100 ppm in chewing gum)
-
Flag: Critical study for SIDS endpoint

10-NOV-2004 (3)

Type: use
Category: Food/foodstuff additives

Remark: flavoring
-
Flag: Critical study for SIDS endpoint
19-MAR-2004 (6)

Type: use
Category: Intermediates

21-SEP-1994

Type: use
Category: Odour agents

Remark: Application: Fragrances
Very useful for cosmetics and especially for lipsticks.
-
Flag: Critical study for SIDS endpoint
10-NOV-2004 (3)

Type: use
Category: Odour agents

Remark: perfumery
-
Flag: Critical study for SIDS endpoint
19-MAR-2004 (6)

Type: use
Category: other: perfume

Remark: Perfumes: Substances which are added to cosmetic products for the purpose of perfume. This includes substances used in fragrances for their properties in mixtures (solvents, excipients, etc.).
Source: EU. Commission Decision 96/335/EC establishing an inventory and a common nomenclature of ingredients employed in cosmetic products. O.J. (L 132) 1, 1 Jun 1996.
From Part II: Perfume and Aromatic Raw Materials.
09-DEC-2003 (15)

1.7.1 Detailed Use Pattern

1.7.2 Methods of Manufacture

Orig. of Subst.: Synthesis
Type: Production

Remark: Ionones, irones, and methylionones, as well as allylionone, are all produced by analogous routes. Special procedures must be used to obtain a particular isomer, either pure or as the main component.
In all processes an acyclic precursor, called a pseudoionone, pseudoirone, etc., is prepared by base-catalyzed condensation

of citral or 6-methylcitral with acetone, methyl ethyl ketone, or allylacetone, as appropriate.
---
beta-ionone can be made from citral and acetone or from dehydrolinalool and diketene or isopropenyl methyl ether via pseudoionone.

Flag: Critical study for SIDS endpoint
02-JUL-2004 (5)

1.8 Regulatory Measures

1.8.1 Occupational Exposure Limit Values

Type of limit: MAK (DE)
Limit value: other: no MAK value established

Country: Germany
Flag: Critical study for SIDS endpoint
13-AUG-2004 (16)

Limit value: other: no workplace control parameters

Flag: Critical study for SIDS endpoint
13-AUG-2004 (13)

1.8.2 Acceptable Residues Levels

1.8.3 Water Pollution

Classified by: other: VwVwS (Germany), Annex 3
Labelled by: other: VwVwS (Germany), Annex 3
Class of danger: 2 (water polluting)

Country: Germany
Remark: ID-Number: 4557
Flag: Critical study for SIDS endpoint
13-AUG-2004 (17)

1.8.4 Major Accident Hazards

Legislation: Stoerfallverordnung (DE)
Substance listed: no

Country: Germany
Flag: Critical study for SIDS endpoint
10-NOV-2004 (18)

1.8.5 Air Pollution

Classified by: TA-Luft (DE)
Labelled by: TA-Luft (DE)
Number: other: 5.2.5. organic gases

Country: Germany
Flag: Critical study for SIDS endpoint
10-NOV-2004 (13)

1.8.6 Listings e.g. Chemical Inventories

Type: EINECS
Additional Info: EINECS No. 201-224-3

Flag: Critical study for SIDS endpoint
09-DEC-2003 (19)

Type: ENCS
Additional Info: ENCS No. 3-2387X

Remark: For ENCS chemical class or category name, refer to ENCS No. 3-2387.
Flag: Critical study for SIDS endpoint
09-DEC-2003 (19)

Type: ECL
Additional Info: ECL Serial No. KE-34479

Flag: Critical study for SIDS endpoint
09-DEC-2003 (19)

Type: TSCA

Flag: Critical study for SIDS endpoint
09-DEC-2003 (19)

Type: PICCS

Flag: Critical study for SIDS endpoint
09-DEC-2003 (19)

Type: DSL

Flag: Critical study for SIDS endpoint
09-DEC-2003 (19)

Type: AICS

Flag: Critical study for SIDS endpoint
09-DEC-2003 (19)

Type: other: EU. Commission Decision 1999/217/EC adopting a register of flavoring substances used in or on foodstuffs
Additional Info: as amended by Decision 2002/113/EC, 2002 OJ (L 49) 1, 20 February 2002. (Part A)

Country: Europe
Flag: Critical study for SIDS endpoint
13-AUG-2004 (15)

Type: other: EU. Commission Decision 96/335/EC establishing an inventory and a common nomenclature of ingredients employed in cosmetic products. O.J. (L 132) 1, 1 Jun 1996.
Additional Info: From Part II: Perfume and Aromatic Raw Materials.

Country: Europe
Flag: Critical study for SIDS endpoint
10-NOV-2004 (15)

Type: other: OECD. Representative List of High Production Volume Chemicals (HPV).

Country: Europe
Flag: Critical study for SIDS endpoint
13-AUG-2004 (15)

Type: other: US Federal, Food and Drugs, FEMA GRAS Listing

Flag: Critical study for SIDS endpoint
10-NOV-2004 (15)

1.9.1 Degradation/Transformation Products

EINECS-Name: No hazardous decomposition products if stored and handled as prescribed/indicated.

Flag: Critical study for SIDS endpoint
17-AUG-2004 (13)

1.9.2 Components

1.10 Source of Exposure

Source of exposure: other: Occurence in nature
Exposure to the: Substance

Remark: Found in raspberries, peaches, passio fruit, melons, grapes, tea.
-
Flag: Critical study for SIDS endpoint
18-MAR-2004 (2)

Source of exposure: other: Occurence in nature
Exposure to the: Substance

Remark: The ionones appearing in many essential oils, in different berries, tea and tobacco just as in flower scents.
They evolve by oxidative degredation of carotines.
-
Flag: Critical study for SIDS endpoint
10-NOV-2004 (4)

Source of exposure: other: Occurence in nature
Exposure to the: Substance

Remark: The C13 ketones alpha- and beta-ionone ... occur in many essential oils.
-
Flag: Critical study for SIDS endpoint
19-MAR-2004 (5)

Source of exposure: Environment: exposure from production
Exposure to the: Substance

Remark: Due to the declaration of the German Emission Register 2000 during production and processing at the BASF AG side less than 25 kg/a were emitted to the atmosphere.
Flag: Critical study for SIDS endpoint
15-AUG-2005 (20)

1.11 Additional Remarks

Memo: Hazardous reactions:
No hazardous reactions if stored and handled as prescribed/indicated.

Flag: Critical study for SIDS endpoint
25-APR-2005 (13)

1.12 Last Literature Search

Type of Search: Internal and External
Chapters covered: 1
Date of Search: 18-MAR-2004

Flag: Critical study for SIDS endpoint
19-MAR-2004

Type of Search: Internal and External
Chapters covered: 8
Date of Search: 18-MAR-2004

Flag: Critical study for SIDS endpoint
19-MAR-2004

Type of Search: Internal and External
Chapters covered: 5.10
Date of Search: 30-APR-2004

Flag: Critical study for SIDS endpoint
30-APR-2004

Type of Search: Internal and External
Chapters covered: 3, 4, 5
Date of Search: 03-JUN-2004

Flag: Critical study for SIDS endpoint
27-SEP-2004

1.13 Reviews

2.1 Melting Point

Value: = -35 degree C

Remark: As solvent petroleum ether was used.
Reliability: (2) valid with restrictions
Peer-reviewed handbook
Flag: Critical study for SIDS endpoint
04-JAN-2006 (21)

Value: = -32 degree C

Reliability: (4) not assignable
Manufacturer / producer data without proof

04-JAN-2006 (13)

2.2 Boiling Point

Value: = 120 degree C at 7 hPa

Reliability: (4) not assignable
original data not available
17-AUG-2004 (2)

Value: = 126 - 128 degree C at 15.99 hPa

Remark: The pressure is indicated with 12 mmHg (corresponds to 1599.64 Pa).
Reliability: (4) not assignable
original data not available
09-NOV-2004 (10)

Value: = 140 degree C at 24 hPa

Reliability: (4) not assignable
original data not available
17-AUG-2004 (4)

Value: = 262.9 degree C

Method: other: calculated
Year: 2005

Reliability: (2) valid with restrictions
scientifically acceptable calculation
Flag: Critical study for SIDS endpoint
04-JAN-2006 (22)

Value: = 267 degree C at 1013 hPa

Reliability: (4) not assignable
Manufacturer / producer data without proof

04-JAN-2006 (13)

2.3 Density

Type: density
Value: = .944 g/cm³ at 20 degree C

Reliability: (4) not assignable
Manufacturer / producer data without proof
20-JAN-2004 (13)

Type: density
Value: = .9447 g/cm³ at 20 degree C

Method: other: measured with double-capillary pycnometers
Year: 1988

Result:

temperature (°C)	density (g/cm3)
20.0	0.9447
30.0	0.9362
40.0	0.9287
50.0	0.9206
60.0	0.9127
70.0	0.9037

Test substance: Beta-Ionone, purity 99.0 mol%
Reliability: (2) valid with restrictions
Scientifically acceptable study, meets basic scientific principles, but without detailed documentation of the employed method
Flag: Critical study for SIDS endpoint
21-JAN-2004 (23)

Type: density
Value: = .9477 g/cm³ at 20 degree C

Test substance: beta-Ionone, purity 99.8 mol%
Reliability: (4) not assignable
secondary quotation
21-JAN-2004 (24)

2.3.1 Granulometry

2.4 Vapour Pressure

Value: = .017 hPa at 25 degree C

Method: other (calculated): SRC MPBPWIN v1.41
Year: 2005

Reliability: (2) valid with restrictions
scientifically acceptable calculation
15-AUG-2005 (22)

Value: ca. .072 hPa at 25 degree C

Method: other (measured): stripping experiments in an aerated stirred

reactor
Year: 1999

Result: The vapour pressure is indicated with 0.072 +/- 0.012 hPa at 25°C.
Test substance: Beta-ionone, indication of origin and purity is missing
Reliability: (2) valid with restrictions
Scientifically acceptable study, although no information on the purity of beta-ionone is given
09-NOV-2004 (25)

Value: = 1 hPa at 83 degree C

Remark: 3 hPa at 100°C
Reliability: (4) not assignable
Manufacturer / producer data without proof
21-JAN-2004 (13)

Value: = 1 hPa at 83.2 degree C

Method: other (measured): dynamic in an argon atmosphere
Year: 1989

Result:

temperature (°C)	vapour pressure (hPa)
83.18	1.00
94.62	2.00
101.80	3.00
111.36	5.00
118.10	7.00
125.51	10.00
140.66	20.00
150.28	30.00
163.38	50.00
172.50	70.00
182.67	100.00
204.36	200.0
218.12	300.0
237.55	500.0
251.5	700.0

Based on these experimental data, the vapour pressure (Pa) at 25°C was calculated based on the following equation: ln (p/bar) = a + b/ (c+t/Celsius), where a = 10.1210, b = -4570.40b and c = 185.09. Therefore, the vapour pressure is 0.9 Pa.
Test substance: CAS 79-77-6,
4-(2,6,6-trimethyl-1-cyclohexene-1-yl)-3-buten-2-one,
beta-ionone, purity: 96.24%
Reliability: (2) valid with restrictions
Comprehensible and acceptable
Flag: Critical study for SIDS endpoint
18-MAR-2004 (26)

Value: = 2.31 hPa at 96.9 degree C

Method: other (measured)
Year: 1982

Remark: Intensive yellow colouration at 215.66°C

Result: temperature (°C) vapour pressure (hPa)

96.85	2.31
110.79	4.91
125.20	10.00
149.94	29.85
178.97	90.02
215.66	300.2
258.59	1000.0

Test substance: Beta-Ionone, purity: 97.96% (m/m); contains 1.45% (m/m) alpha-ionone
Reliability: (2) valid with restrictions
Comprehensible and acceptable
18-MAR-2004 (27)

Value: = 6.8 hPa at 100.3 degree C

Method: other (measured): static method with a glass membrane as a null manometer

Result: temperature (°C) vapour pressure (hPa)

100.3	6.8
106.8	8.1
111.8	9.9
115.4	11.1
119.7	12.0
126.0	15.7
131.5	20.3
138.0	25.1
144.6	32.0
153.8	45.3
157.1	50.1
163.2	63.1
169.1	77.1

Test substance: 4-(2,6,6-trimethyl-1-cyclohexene-1-yl)-3-buten-2-one, beta-ionone, purity 99.0 mol%
Reliability: (2) valid with restrictions
Scientifically acceptable study, meets basic scientific principles, but without detailed documentation of the employed method
09-NOV-2004 (28)

2.5 Partition Coefficient

Partition Coeff.: octanol-water
log Pow: = 4 at 25 degree C

Method: other (measured): HPLC method according to EEC Directive 79/831, Annex V, Part A
Year: 1989
GLP: no

Test substance: 4-(2,6,6-trimethyl-1-cyclohexenyl)-3-buten-2-one, beta-ionone; purity 95-98 % of the trans-isomer (see also chapter 1.1.2)
Reliability: (2) valid with restrictions
according to guideline, non GLP
Flag: Critical study for SIDS endpoint
09-NOV-2004 (29)

Partition Coeff.: octanol-water
log Pow: = 4.1 at 24 degree C

Method: OECD Guide-line 117 "Partition Coefficient (n-octanol/water), HPLC Method"
Year: 1994
GLP: yes

Test substance: Beta-Ionone (CAS: 14901-07-6); purity: 97.0 % of the trans-Isomer (see also chapter 1.1.2)
Reliability: (1) valid without restriction
Guideline study
16-AUG-2004 (30)

Partition Coeff.: octanol-water
log Pow: = 4.4 at 25 degree C

Method: other (calculated): via SRC KOWWIN v1.66
Year: 2003

Reliability: (2) valid with restrictions
Scientifically acceptable method
18-JUN-2004 (31)

2.6.1 Solubility in different media

Solubility in: Water
Value: = .128 g/l at 25 degree C

Reliability: (4) not assignable
Manufacturer / producer data without proof
20-JAN-2004 (13)

Solubility in: Water
Value: = .169 g/l at 25 degree C

Method: other: measured
Year: 1999

Method: The author indicates that the solubility was determined by measuring solute content in a saturated aqueous solution obtained using a two-liquid-phase contact method at a given temperature.

No further details are given.
Remark: The solubility is indicated as 0.88+/- 0.08 mmol/l at 25°C.
Test substance: Beta-ionone, indication of purity is missing
Reliability: (2) valid with restrictions
Scientifically acceptable study, although no information on the purity of beta-ionone is given
Flag: Critical study for SIDS endpoint
15-AUG-2005 (25)

2.6.2 Surface Tension

Test type: other: capillary method
Value: = 39.52 mN/m at 20 degree C

Method: other: measured
Year: 1988

Remark: The results refer to the neat liquid.
Result:

temperature (°C)	surface tension (mN/m)
20.0	39.52
30.0	38.43
40.0	37.24
50.0	36.11
60.0	34.94
70.0	33.77

Test substance: 4-(2,6,6-trimethyl-1-cyclohexene-1-yl)-3-buten-2-one, beta-ionone, purity 99.0 mol%
Reliability: (2) valid with restrictions
Scientifically acceptable study, meets basic scientific principles, but without detailed documentation of the employed method
Flag: Critical study for SIDS endpoint
21-JAN-2004 (28)

2.7 Flash Point

Value: = 127 degree C
Type: closed cup

Method: other: German Industrial Standard DIN 51758
Year: 1989

Test substance: Beta-ionone, indication of purity is missing
Reliability: (2) valid with restrictions
scientifically comprehensible, method used is indicated
Flag: Critical study for SIDS endpoint
12-NOV-2004 (32)

2.8 Auto Flammability

Value: = 270 degree C

Method: other: German Industrial Standard DIN 51794

Remark: Inflammation temperature
Test substance: Beta-ionone, indication of purity is missing
Reliability: (2) valid with restrictions
scientifically comprehensible, method used is indicated
Flag: Critical study for SIDS endpoint
15-AUG-2005 (32)

2.9 Flammability

24-NOV-1997

2.10 Explosive Properties

Result: not explosive

Remark: The substance has no chemical groups indicating explosive properties
Test substance: (E)-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-3-buten-2-one; CAS: 79-77-6
Reliability: (2) valid with restrictions
Expert judgement
Flag: Critical study for SIDS endpoint
15-JAN-2001 (33)

2.11 Oxidizing Properties

Result: no oxidizing properties

Remark: The substance has no chemical groups indicating oxidizing properties
Test substance: (E)-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-3-buten-2-one; CAS: 79-77-6
Reliability: (2) valid with restrictions
Expert judgement
Flag: Critical study for SIDS endpoint
21-JAN-2004 (33)

2.12 Dissociation Constant

2.13 Viscosity

Value: = 10.8 mPa s (dynamic) at 20 degree C

Remark: Reason for flagging this study: only study available for this endpoint
Test substance: Beta-ionone, indication of purity is missing
Reliability: (4) not assignable
Manufacturer / producer data without proof
Flag: Critical study for SIDS endpoint
13-FEB-2004 (13)

2.14 Additional Remarks

Remark: Explosion limits:
lower limit: 1 vol.%
upper limit: 5.6 vol.%
Test substance: Beta-ionone, indication of purity is missing
Reliability: (4) not assignable
Manufacturer / producer data without proof
15-JAN-2001 (13)

Method: Compensation method
Remark: Heat capacity
Result: temperature (°C) heat capacity (g/cm3)

0.1	1.89
20.7	1.95
40.5	2.02
60.6	2.05

Test substance: 4-(2,6,6-trimethyl-1-cyclohexene-1-yl)-3-buten-2-one, beta-ionone, purity 99.0 mol%

Reliability: (2) valid with restrictions
Scientifically acceptable study, meets basic scientific principles, but without detailed documentation of the employed method

21-JAN-2004 (28)

Remark: Thermally stable at temperatures up to 200°C
Test substance: Beta-ionone, indication of purity is missing
Reliability: (4) not assignable

23-JAN-2004 (13)

Method: The affinity of bioactive compounds for lipid matrices was studied using a quartz-crystal microbalance (QCM) coated with lipid multilayers. The QCM immersed into aqueous solutions of the test compounds. The frequency decrease (mass increase) caused by test compound deposited onto the lipid matrix was followed using an oscillator connected to a microcomputer. Partition coefficients from the aqueous phase to the lipid matrix were calculated by dividing the amount adsorbed onto the lipid matrix by the concentration in the aqueous phase.

Result: Beta-ionone adsorbed specifically onto lipid bilayer matrices. Partition coefficients (P) of beta-ionone for membrane-coated QCMs at 45°C were:

Type of membrane on QCM	P	log P
uncoated	10	1
C18N+2C1/PSS-film (lipid bilayer)	3050	3.48
DMPE (naturally occurring phospho-lipid multibilayer)	2700	3.43
polystyrene	35	1.54
bovine plasma albumin	30	1.47
keratin	30	1.47

Test substance: Beta-ionone, indication of purity is missing
Reliability: (2) valid with restrictions
Scientifically acceptable, well documented and comprehensible

03-FEB-2004 (34)

3.1.1 Photodegradation

Type: air
INDIRECT PHOTOLYSIS
Sensitizer: O3
Conc. of sens.: 700000000000 molecule/cm³
Rate constant: = .000000000000009240075 cm³/(molecule * sec)
Degradation: = 50 % after 17.9 minute(s)

Method: other (calculated): using SRC AOP v1.90
Year: 2003

Reliability: (2) valid with restrictions
Scientifically acceptable method
Flag: Critical study for SIDS endpoint
21-MAR-2004 (35)

Type: air
INDIRECT PHOTOLYSIS
Sensitizer: OH
Conc. of sens.: 1500000 molecule/cm³
Rate constant: = .0000000002385475 cm³/(molecule * sec)
Degradation: = 50 % after .5 hour(s)

Method: other (calculated): using SRC AOP v1.90
Year: 2003

Test condition: Calculated t1/2 based on a 12 hour day
Reliability: (2) valid with restrictions
Scientifically acceptable method
21-MAR-2004 (35)

Type: air
INDIRECT PHOTOLYSIS
Sensitizer: OH
Conc. of sens.: 500000 molecule/cm³
Rate constant: = .0000000002385475 cm³/(molecule * sec)
Degradation: = 50 % after 1.6 hour(s)

Method: other (calculated): using SRC AOP v1.90
Year: 2003

Test condition: Calculated t1/2 based on a 24 hour day
Reliability: (2) valid with restrictions
Scientifically acceptable method
Flag: Critical study for SIDS endpoint
21-MAR-2004 (36)

3.1.2 Stability in Water

Remark: Due to the chemical structure beta-ionone is not expected to undergo hydrolysis.
Reliability: (2) valid with restrictions
Expert Judgement, scientifically acceptable
16-NOV-2004 (37)

3.1.3 Stability in Soil

Remark: Beta-ionone is readily and fast biodegradable and is therefore expected to transform rapidly in soil.
Reliability: (2) valid with restrictions
Expert Judgement, scientifically acceptable
15-AUG-2005 (37)

3.2.1 Monitoring Data (Environment)

Type of measurement: concentration at contaminated site
Medium: ground water
Concentration: = .028 - .655 µg/l
Method: Enrichment on charcoal or Amberlite XAD-2; detection and quantification (internal standard anthraen-d10) with GC/MS

Result: In the Besós area in N.E. Spain, gravel was extracted to under phreatic levels. Refilling of the pits with industrial and domestic waste led to contamination of the groundwater. A trace organic survey was carried out to determine the extent of groundwater pollution and to evaluate whether the contaminants could be removed by filtering with charcoal. For enrichment of organic pollutants water samples (200 l) were passed (1) through granular activated carbon (GAC) and (2) through Amberlite XAD-2 resin.

The following concentrations of ionone were measured in raw and filtered water:
(1) raw water: 125-655 ng/l
water passed though GAC: 5 ng/l
(2) raw water: 28-535 ng/l
water passed through XAD-2: 5 ng/l
Test substance: Ionone; no further specification is provided.
Reliability: (2) valid with restrictions
Scientifically acceptable method, meets basic scientific principles
12-NOV-2004 (38)

Type of measurement: concentration at contaminated site
Medium: surface water
Method: concentration analysis via GC/MS

Remark: The elimination of odorous compounds by river bank filtration of a reservoir was studied. 10 l water samples from different observation wells were taken. The analysis was undertaken not more then 12 h later in a stripping advice for separation of VOC's using GC/MS. The quantitaive analyses were made in single ion monitoring mode after calibaration where reference compounds were used.
Result: In the river water 1.8 ng/l beta-Ionon was found. In the river bank filtrated water only 0.13 ng/l and 0.31 ng/l could be determined, respectively. Both values show a high elimination rate of 83 % and 93 % by passing the river bank, respectively.
Test substance: Beta-ionone, indication of CAS number and purity is missing
Reliability: (2) valid with restrictions
Scientifically acceptable method
12-NOV-2004 (39)

Type of measurement: concentration at contaminated site
Medium: other: surface water
Method: GC/MS (after SPE and CLSA)

Remark: For identification and quantification of traces of odorous compounds in surface waters solid phase extraction (SPE) and closed loop stripping- analysis (CLSA) methods were optimised. Beta-Ionon was one compound analysed by GC/MS-screening. Additionally, odour threshholds were determined.
Result: The odour threshold of beta-Ionon was 1 µg/l (subjective determination by smelling at different days).
In the water of the river Ruhr (near Echthausen) 0.005 µg/l beta-Ionon was found via GC/MS using SPE and CLSA techniques (quantification limit 0.002 µg/l, recovery rate 94 %). This value is lower then the odour threshold.
Test substance: Beta-ionone, indication of CAS number and purity is missing
Reliability: (2) valid with restrictions
Scientifically acceptable method
Flag: Critical study for SIDS endpoint
19-NOV-2004 (40)

Type of measurement: other: concentration in leaf abrasion products
Medium: other: aerosol
Method: GC/MS

Result: Green and dead leaves from 62 plant species characteristic of the Los Angeles area were harvested and composited according to the actual leaf mass distribution of the area. To simulate leaf surface abrasion, leaf composites were agitated in Teflon bags with a purified air stream flow. Fine particles (<2 µm) shed from the leaf surfaces were collected on quartz fibres and extracted by mild ultrasonic agitation in hexane and benzene/2-propanolol. The extract was evaporated and compounds were analysed by GC/MS. Beta-ionone content was 95.4 µg/g of green leaf abrasion products and 13.6 µg/g of dead leaf abrasion products (identification according to library spectrum verification).
Reliability: (2) valid with restrictions
Scientifically acceptable method
09-NOV-2004 (41)

Type of measurement: other: concentration in red wine
Medium: food
Concentration: = .72 - µg/l

Result: Red wine (Grenache wine, Spain) was extracted with freon, concentrated and analysed by GC/MS (quantification: comparison with internal standard, identification: not stated). The concentration of beta-ionone was 0.72 µg/l.
Reliability: (2) valid with restrictions
Scientifically acceptable method
Flag: Critical study for SIDS endpoint
09-NOV-2004 (42)

Type of measurement: other: concentration in tobacco plants (Nicotiana tabacum)
Medium: biota
Method: GC/MS

Method: Changes in levels of endogenous beta-ionone in tissues of

tobacco following injection of viable Peronospora tabacina sporangia were investigated. Tobacco plants were sampled 3-5 weeks after injection of P. tabacina. Volatile constituents were obtained by steam distillation/continuous solvent extraction of frozen leaf and stem tissue and identified by GC/MS (identification: comparison with retention time and mass spectrum of authentic b-ionon, quantification: int. standard n-decanol).

Result: Beta-ionone content in green tissues of stems of control plants and in tissue from necrotic lesions of plants injected with P. tabacina was <10 ng/g fresh weight. Beta-ionone content in green tissues of stems of plants injected with P. tabacina ranged from ca. 500 to 6000 ng/g fresh weight.

Reliability: (2) valid with restrictions
Scientifically acceptable method

Flag: Critical study for SIDS endpoint

09-NOV-2004 (43)

Type of measurement: other: concentrations in lake water
Medium: surface water
Concentration: ca. .025 - .44 µg/l
Method: GC/MS

Result: Volatile substances present in water of Lake Federsee, a shallow unstratified eutrophic lake in SW Germany, were determined. Water samples were taken every 2 weeks (May-Nov.) from the middle of the lake at a depths of ca. 20 cm. Following addition of NaCl, the volatile components were stripped from the water in a closed loop system, trapped on a solid adsorbent (Tenax GC) and determined by GC/MS (identification and quantification: reference compound). The beta-ionone concentration in L. Federsee water was ca. 25-50 ng/l in May. It increased to ca. 440 ng/l in the beginning of June and then dropped again and remained ca. 25 ng/l and below between mid-June and November (all concentrations were read from a graph).

Reliability: (2) valid with restrictions
Scientifically acceptable, well documented and comprehensible

Flag: Critical study for SIDS endpoint

16-NOV-2004 (44)

Type of measurement: other: concentrations in lake water and bank filtrate
Medium: other: surface water and bank filtrate
Concentration: ca. 0 - .025 µg/l
Method: GC/MS

Remark: Results are semiquantitative and represent minimal concentrations as the stripping/purging method removes the organics incompletely from the water.

Result: The composition of volatile organic compounds was studied in surface water and bank filtrate from three lakes with different trophic state in Berlin (Germany). Lake water was sampled at 2 m depth and, during thermal stratification, above ground. Volatile organic compounds were stripped (addition of NaCl, closed-loop stripping system), adsorbed onto Tenax traps, thermally desorbed and analysed by GC/MS. Beta-ionone was detected in concentrations of up to ca. 25 ng/l in Lake Tegel, where it appeared during the decline of

central diatoms in spring. In Lake Wannsee, beta-ionone was detected in concentrations of up to ca. 25 ng/l in late summer and early autumn. Beta-ionone was neither detected in the mesotrophic Lake Schlachtensee nor in bank filtrate of the three lakes.
(Detection limit 1 ng/l, identification: measurement of standards, quantification: external standard)

Reliability: (2) valid with restrictions
Scientifically acceptable method

Flag: Critical study for SIDS endpoint

09-NOV-2004 (45)

Type of measurement: other: concentrations in river and reservoir water
Medium: surface water
Concentration: ca. .05 - 1.2 µg/l
Method: GC/MS

Result: The production of volatile odour compounds by freshwater phytoplankton was monitored weekly from Nov. to April at two sites in New South Wales, Australia: (1) Hay Weir Pool, Murrumbridgee River (a typical lowland river) and (2) Carcoar Dam (a headwater reservoir used for water supply). Surface water samples were preserved with HgCl2. Volatile organic compounds were extracted by closed loop stripping and quantified by GC/MS.
The beta-ionone concentrations in Hay Weir Pool ranged from ca. 50 to ca. 1200 ng/l (concentrations were read from a graph).
There was a significant correlation between abundance of Anabaena sp. and beta-ionone concentrations ($p<0.01$, Spearman correlation analysis). A mean concentration of 20 fg of beta-ionone per Anabaena cell was calculated.
No significant correlation was found between concentrations of beta-ionone and the abundance of Anabaena sp. at Carcoar Dam.
(Detection limit estimatet from graph < 5 ng/l, identification: coelution of standards, quantification: internal standard)

Reliability: (2) valid with restrictions
Scientifically acceptable method

Flag: Critical study for SIDS endpoint

09-NOV-2004 (46)

Type of measurement: other: concentrations in river water
Medium: surface water
Concentration: = .002 - .007 µg/l
Method: GC/MS

Result: Water samples were collected in River Arno (Italy) in May 1995 during an off-flavour period at 7 locations near Florence. Following the addition of NaCl, water samples were extracted (microextraction with hexane). The extracts were analysed by gas chromatographic-ion-trap detection-mass spectrometry (GC-IDT-MS). The beta-ionone was detected at 6 out of the 7 locations. The measured concentrations ranged from 2 to 7 ng/l.
(Recovery rate 99%, detection limit 1 ng/l, identification: measurement of standards, quantification: calibration curves from spiced samples)

Reliability: (2) valid with restrictions

Scientifically acceptable method
09-NOV-2004 (47)

Type of measurement: other: emission from Boronia megastigma (Rutaceae)
Medium: biota
Method: Solid phase microextraction (SPME) and GC/MS

Result: Emission of volatiles by brown boronia (Boronia megastigma) was investigated. Solid phase microextraction (SPME) was used to trap volatiles in the headspace above buds, flowers throughout maturation, dissected floral organs and above whole plants held for 36 hours under different light regimes. Volatiles were desorbed and analysed by GC/MS. Beta-ionone was the major volatile emitted from B. megastigma.
(Identification: comparison with known retention times/Kovacs indices, quantification: peak areas)

	Beta-ionone (% of volatiles in headspace)
very small buds	5
medium buds	3
large buds	55
open flowers	40
senescent flowers	13
calyx/nectary	30
petals	68
stigma	88
sepaline anthers	34
petaline anthers	58

Emission of beta-ionone was enhanced in the dark.
Reliability: (2) valid with restrictions
Scientifically acceptable method
Flag: Critical study for SIDS endpoint
16-NOV-2004 (48)

Type of measurement: other: emission from broccoli
Medium: food
Method: Capillary GLC-MS

Result: Volatiles were isolated from broccoli (Brassica oleracea var. Italica) stored under controlled atmospheres containing different levels of nitrogen, oxygen and carbon dioxide. Volatiles were trapped on Tenax GC and analysed by capillary GLC-MS.

		Atmospheric condition			
Concentration of beta-ionone	air	0.5% O2 20% CO2	0.5% O2 0% CO2	0% O2 20% CO2	100% N2
(ppb)	0.1	2	2	3	7
(µg/m3)	0.8	16	16	24	56

(Identification with authentic substance)
Reliability: (2) valid with restrictions
Scientifically acceptable method

09-NOV-2004 (49)

Type of measurement: other: emission from maize kernels

Medium: food
Method: GC/MS

Result: Headspace volatiles from ground maize kernels from the basal, medial and apical zone of maize ears were sampled and analysed by GC/MS.
The emission of beta-ionone from maize kernels was as follows:
Apical zone: 12.8+/-1.6 ng/h
Medial zone: 15.3+/-2.1 ng/h
Basal zone: 0

(Identification and quantification with authentic substances)
Reliability: (2) valid with restrictions
Scientifically acceptable method
Flag: Critical study for SIDS endpoint
09-NOV-2004 (50)

Type of measurement: other: in Boronia megastigma (Rutaceae)
Medium: biota
Method: GC

Result: Brown boronia (Boronia megastigma), an endemic Tasmanian shrub, is used for production of a floral extract.
Beta-ionone is one of the main volatiles of B. megastigma (1.2-4.5% of the extract). The effect of postharvest incubation on the concentration of volatiles in boronia flowers was investigated. Following incubation, flowers were frozen. After thawing, they were extracted and volatiles were analysed by GC.
Concentrations of beta-ionone ranged from ca. 0.015 to 0.03% of the thawed flower weight.
Reliability: (2) valid with restrictions
Scientifically acceptable method
Flag: Critical study for SIDS endpoint
09-NOV-2004 (51)

Type of measurement: other: in Boronia megastigma (Rutaceae)
Medium: biota
Method: GC/MS

Result: The relative amount of volatile compounds in extracts and headspace from different floral organs were assessed. Floral organs were extracted using hexane while headspace samples were injected onto a cryotrap. Volatiles in extracts and headspace were identified by GC/MS.
Beta-ionone accounted for ca. 8% of the volatiles in the extracts from calyx and sepals, for ca. 40% of the volatiles in the headspace of the petals and for ca. 55% of the volatiles in the headspace of calyx and sepals.
Reliability: (2) valid with restrictions
Scientifically acceptable method
Flag: Critical study for SIDS endpoint
09-NOV-2004 (52)

Type of measurement: other: in Boronia megastigma (Rutaceae)
Medium: biota
Method: Gas liquid chromatography

Result: Variation in the oil composition of the flowers from 29

different populations of Boronia megastigma was investigated. Flowers were extracted with ethanol and the extract was analyzed by gas liquid chromatography.
Mean content of beta-ionone in the flowers from the different populations ranged from ca. 17 to ca. 1787 µg/g fresh weight of the flowers.

Reliability: (2) valid with restrictions
Scientifically acceptable method
Flag: Critical study for SIDS endpoint
09-NOV-2004 (53)

Type of measurement: other: in Boronia megastigma (Rutaceae)
Medium: biota
Method: GC

Result: The effect of the form of nitrogen fertiliser (isobutylidene [IBDU], urea, calcium nitrate and ammonium nitrate) and the application rate (25, 50 and 100 kg N/ha) on beta-ionone content of the oil of B. megastigma was investigated. Flowers were extracted using hexane and petroleum ether, the solvent was evaporated and beta-ionone content in the so-called 'concrete' was measured by GC.

Content of beta-ionone	Rate of application (kg N/ha) 25	50	100
% of volatiles	34.53	34.74	34.95
% of 'concrete'	2.73	3.06	3.67

Content of beta-ionone	Nitrogen form IBDU	urea	calcium nitrate	ammonium nitrate
% of volatiles	34.48	34.87	35.06	34.87
% of 'concrete'	2.86	3.10	3.24	3.42

Reliability: (2) valid with restrictions
Scientifically acceptable method
Flag: Critical study for SIDS endpoint
09-NOV-2004 (54)

Type of measurement: other: in Boronia megastigma and B. heterophylla (Rutaceae)
Medium: biota
Method: Gas liquid chromatography

Result: The influence of light intensity on volatile oil composition in Boronia megastigma was investigated. Flowers were extracted in ethanol and volatiles were identified using gas liquid chromatography.
Beta-ionone content increased with decreasing light intensity.

Sunlight (%)	beta-ionone (µg/g fresh weight)
100	128
75	178
39	262
27	394
11	334

Reliability: (2) valid with restrictions
Scientifically acceptable method
Flag: Critical study for SIDS endpoint
09-NOV-2004 (55)

Type of measurement: other: in Cerastium candidissimum (Caryophyllaceae)
Medium: biota
Method: GC - electron impact MS

Result: The volatile constituents of the aerial parts of Cerastium candidissimum (Caryophyllaceae) were extracted by steam distillation and analyzed by GC - electron impact MS.
Trans-beta-ionone accounted for 13.4% of the volatile constituents of C. candidissimum.
Reliability: (2) valid with restrictions
Scientifically acceptable method
Flag: Critical study for SIDS endpoint
09-NOV-2004 (56)

Type of measurement: other: in Cistus monspeliensis (Cistaceae)
Medium: biota
Method: GC/MS

Result: Powdered leaves and fruits of Cistus monspeliensis were extracted using hexane, purified by column chromatography and analyzed by GC/MS. In addition, oil was extracted from both leaves and fruits by hydrodistillation. The oil was dried and analyzed by GC/MS.
Beta-ionone accounted for 2.07% of the essential oil of the leaves of C. monspeliensis, but it was not found in essential oil of fruits nor in hexance extract of leaves and fruits.
Reliability: (2) valid with restrictions
Scientifically acceptable method
Flag: Critical study for SIDS endpoint
09-NOV-2004 (57)

Type of measurement: other: in Lepidium meyenii (Brassicaceae)
Medium: biota
Method: GC/MS

Result: The essential oil profile of maca (Lepidium meyenii, Brassicaceae) from Lima, Peru, was investigated. Steam distillates of the aerial parts of L. meyenii were extracted with pentane and the extracts were analyzed by GC/MS.
E-beta-ionone was one of the constituents of L. meyenii-oil (0.1% of the relative peak area).
Reliability: (2) valid with restrictions
Scientifically acceptable method
Flag: Critical study for SIDS endpoint
09-NOV-2004 (58)

Type of measurement: other: in Medicago marina (Fabaceae)
Medium: biota
Method: GC/MS

Result: The essential oils of Medicago marina were obtained by hydrodistillation of the dried, crushed aerial parts of the plant and analysed by GC/MS.
(E)-beta-ionone accounted for 14.4% and 5.6% of the essential oil constituents of plants sampled during the vegetative and flowering phase, respectively.
Reliability: (2) valid with restrictions
Scientifically acceptable method
Flag: Critical study for SIDS endpoint

02-JUL-2004 (59)

Type of measurement: other: in Osmanthus fragrans (Oleaceae)
Medium: biota
Method: GC/MS

Result: Osmanthus fragrans flowers were extracted with isopentane. The extract was concentrated and analysed by GC/MS. Beta-ionone was detected in the extract (3.38% of the peak area).
Reliability: (2) valid with restrictions
Scientifically acceptable method
Flag: Critical study for SIDS endpoint
13-FEB-2004 (60)

Type of measurement: other: in Rosa bourboniana (Rosaceae)
Medium: biota
Method: GC/MS

Result: Oil of Rosa bourboniana was obtained by hydro-distillation of flowers. It was analysed by GC/MS. The oil contained 0.03% of beta-ionone.
Reliability: (4) not assignable
Article in a popular scientific journal without detailed documentation of methods and results
Flag: Critical study for SIDS endpoint
11-FEB-2004 (61)

Type of measurement: other: in Thymus serpyllum (Labiatae)
Medium: biota
Method: GC

Result: Beta-ionone content was determined in full herb, leaves and flowers from wild thyme (Thymus serpyllum) sampled at three locations in the NW Himalayas. Essential oils were analysed by GC.
The essential oil of the full herb of T. serpyllum contained 0.6-2.1% (w/w) of beta-ionone, leaves and flowers contained 0.3% of beta ionone.
Reliability: (2) valid with restrictions
Scientifically acceptable method although description of methods and results is very brief
Flag: Critical study for SIDS endpoint
13-FEB-2004 (62)

Type of measurement: other: occurrence in a cyanobacterial mat community
Medium: biota
Method: GC/MS

Result: Volatile organic chemicals produced by a cyanobacterial mat community inhabiting a salt-encrusted beach on Wells Lake, a hypersaline lake in Saskatchewan (Canada) were investigated. The community consisted of mat-forming and littoral strains of Oscillatoria animalis and O. subbrevis, other microorganisms associated with these cyanobacteria, several species of beetle (Carabidae, Coleoptera) and halophytic flowering plants.
Samples from salt incrustations and the underlying cyanobacterial layer, cyanobacterial flakes and crusts, halophytic plants and water in shallow pools and lagoons

were taken. Samples were purged with nitrogen, trapped on Tenax GC and analysed by GC/MS.
Beta-ionone was detected in (1) salt incrustations and the underlying cyanobacterial layer, (2) cyanobacterial flakes and crusts, (3) Oscillatoria mats in dried shallow depression and in (4) salt meadow grass (Puccinellia nuttalliana). Concentrations are not indicated.

Reliability: (2) valid with restrictions
Scientifically acceptable method

Flag: Critical study for SIDS endpoint

13-FEB-2004 (63)

Type of measurement: other: occurrence in algae
Medium: biota
Method: GC/MS

Result: Four algal species that had previously been associated with taste and odour episodes in drinking water supplies were cultured: Asterionella formosa, Synedra delicatissima and Aulacosiera granulata (Bacillariophyceae) and Scenedesmus subspicatus (Chlorophyceae). Non-axenic algal monocultures were grown to simulate bloom conditions. Cells were separated from medium by filtration (0.45 µm) and both extracellular and intracellular concentrations of odorous metabolites were determined. Intracellular compounds were released by ultrasonication. Closed loop stripping, open stripping and steam distillation extraction were used to extract and concentrate the metabolites. The compounds were identified by a combination of GC/MS and sensory analyses (sensory GC and flavour profile analysis).
Beta-ionone was one of the constituents contributing to the odour of S. subspicatus. (No information on concentrations in cells or culture water is provided.)

Reliability: (2) valid with restrictions
Scientifically acceptable method

Flag: Critical study for SIDS endpoint

16-FEB-2004 (64)

Type of measurement: other: occurrence in algae
Medium: biota
Method: GC/MS

Result: The odorous compounds of various axenic strains of Anabaeba and Nostoc (Anabaena PCC 7120 [PCC = Pasteur Culture Collection], A. variabilis, A. oscillarioides, Nostoc PCC 6310, N. PCC 6314) were studied. After addition of NaCl cyanobacterial suspensions were stripped in a closed loop system. The odorous compounds were adsorbed onto Tenax GC, thermally eluted and determined by GC/MS.
Beta-ionone was not detected in the investigated strains.

Reliability: (2) valid with restrictions
Scientifically acceptable method

Flag: Critical study for SIDS endpoint

16-NOV-2004 (65)

Type of measurement: other: occurrence in algae
Medium: biota
Method: GC/MS

Result: Microcystes aeruginosa was cultured in the presence of (a) 1

µM FeCl3 (standard medium) and (b) additional 30 µM Fe as ferric citrate. Cultures were exposed for 3, 6 or 9 h to (1) standard artificial illumination or (2) sunlight. Exposure to sunlight led to a significant temperature increase. Volatile organic compounds were extracted by closed loop stripping and analysed by GC/MS.
Concentrations of beta-ionone in M. aeruginosa at low iron conditions in the different light regimes were 0.8, 0.05 and 0.54 µg geosmin equivalents/g dry weight after 3, 6 and 9 h sunlight and 0.08, 0.88 and 5.16 after 3, 6 and 9 h standard artificial illumination.
At high iron conditions, 0, 0.45 and 0 µg geosmin equivalents/g dry weight were detected after 3, 6 and 9 h sunlight and 2.37, 0.83 and 0 µg geosmin equivalents/g dry weight after 3, 6 and 9 h standard artificial illumination.

Reliability: (2) valid with restrictions
Scientifically acceptable method

Flag: Critical study for SIDS endpoint

09-NOV-2004 (66)

Type of measurement: other: occurrence in fish tissue
Medium: biota
Method: GC/MS

Result: Samples of adult lake trout (Salvelinus namaycush) and walleye (Stizostedion v. vitreum) were collected in 1977 from each of the Great Lakes and Lake St. Clair. Lake trout from a hatchery served as control. At each sampling site, 20 trout or walleye were collected. Composited samples of whole fish were prepared by homogenising. Organic contaminants were extracted, separated from lipids by gel permeation chromatography, separated into two fractions on a silica gel column and analysed by GC/MS.
Beta-ionone was detected in fish caught in three of the nine investigated lakes: (1) in lake trout from Lake Huron, Huron, (2) from Lake Michigan and (3) from Lake Superior.

Reliability: (2) valid with restrictions
Scientifically acceptable method

09-NOV-2004 (67)

Type of measurement: other: occurrence in kiwi fruit flowers
Medium: biota
Method: Capillary gas chromatography and GC/MS

Result: The volatile components of kiwi fruit flowers (Actinidia chinensis Planch.) were concentrated by (1) simultaneous steam distillation/solvent (dichloromethane) extraction (SDE) and (2) dynamic headspace sampling and measured by GC/MS.
Using the first method, beta-ionone was identified as a volatile constituent of kiwi fruit flowers (0.04% of the peak area).

Reliability: (2) valid with restrictions
Scientifically acceptable method

03-FEB-2004 (68)

Type of measurement: other: occurrence in river sediments
Medium: sediment
Method: GC/MS

Result: Sediment samples of River Havel and R. Spree (NE Germany) were collected using Eckman-Birge grab samplers or a liquid nitrogen deep freeze method. Organic compounds were extracted and determined by GC/MS (SIM mode). Beta-ionone was commonly observed in sediments of R. Havel and R. Spree (no further details on occurrence and concentrations are provided).
Reliability: (2) valid with restrictions
Scientifically acceptable method
16-FEB-2004 (69)

Type of measurement: other: occurrence in several types of meat
Medium: food

Remark: beta-Ionone isomers occurs as volatiles in beef flavour. (medium and small peak size; no more information on concentration)
(Original references: Peterson et al., 1975; Peterson R.J. and Chang S.S., 1982)
Reliability: (2) valid with restrictions
Scientifically acceptable review
Flag: Critical study for SIDS endpoint
09-NOV-2004 (70) (71) (72)

Type of measurement: other: occurrence in sludge-press liquors
Medium: other: wastewater
Method: GC

Result: Sludge-press liquor samples were taken from the start, middle and end of a sludge press in the sludge-pressing plant of a wastewater treatment works in Canterbury (England). After ozonation, ionone was detected in starting, middle and final sludge-press liquor. (Isomer not specified).
Reliability: (4) not assignable
Extended abstract, no specification of the type of ionone and no information on measured concentrations are provided
09-NOV-2004 (73)

Type of measurement: other: occurrence in small rivers and brooks
Medium: surface water
Method: GC/MS

Result: The volatile organic compounds in 8 weakly polluted small rivers and brooks in SW Germany were studied by GC/MS. Beta-ionone was found in 5 out of the 8 investigated small rivers/brooks (measured concentrations are not indicated).
Reliability: (2) valid with restrictions
Scientifically acceptable method
Flag: Critical study for SIDS endpoint
16-NOV-2004 (74)

3.2.2 Field Studies

3.3.1 Transport between Environmental Compartments

Type: adsorption
Media: water - soil
Method: other: calculated using SRC PCKOCWIN v1.66

Year: 2003

Result: Koc = 625; logKoc = 2.80
Reliability: (2) valid with restrictions
Scientifically acceptable method, model accepted by US EPA
09-NOV-2004 (75)

Type: adsorption
Media: water - soil
Method: other: calculated according to TGD
Year: 2003

Remark: Beta-Ionone has a water solubility of 0.169 mg/l and is a hydrophobic substance. Therefore, the model equation for hydrophobic substances is used.
Result: The calculation was carried out according to TGD (2003), part III, p. 26: based on the equation logKoc = 0.81 * logKow + 0.10 (from Sabljic and Guesten (1995) cited in TGD, 2003) for so-called predominantly hydrophobic substances. Using the measured logKow of 4.1, a logKoc of 3.34 (Koc=2630) was calculated.
Reliability: (2) valid with restrictions
Scientifically acceptable method that is recommended in the TGD
Flag: Critical study for SIDS endpoint
15-AUG-2005 (76)

Type: volatility
Media: water - air
Method: other: calculated
Year: 2003

Result: The calculation was carried out according to Thomas et al, 1982): Henry's law constant H = vapour pressure (Pa)/solubility (mol/m^3).
The vapor pressure vp = 0.9 Pa (25°C) extrapolated from the measured data (see chapter 2.4) and the water solubility ws = 0.88 mol/m^3 (0.169 g/L; 25°C) measured by Fichan et al., 1999 (see chapter 2.6.1) were used.
The resulting Henry's law constant is 1.023 $Pa*m^3/mol$. This indicates a moderate volatility of beta-ionone.
Reliability: (2) valid with restrictions
Scientifically acceptable method
Flag: Critical study for SIDS endpoint
09-NOV-2004 (77)

Type: volatility
Media: water - air
Method: other: calculated using SRC HENRYWIN v3.10
Year: 2003

Result: Henry's Law Constant H = 17.63 $Pa*m^3/mol$
Reliability: (2) valid with restrictions
Scientifically acceptable method
Flag: Critical study for SIDS endpoint
21-MAR-2004 (78)

3.3.2 Distribution

Media: air - biota - sediment(s) - soil - water
Method: Calculation according Mackay, Level I
Year: 2003

Remark: The calculation is based on the following physical and chemical properties:
molecular mass (g/mol): 192.3
log Kow: 4.00 (measured)
water solubility (g/m³): 169 (measured)
vapour pressure (Pa): 0.9 (calculated based on measured data, see chapter 2.4)
melting point: -32°C
data temperature: 25°C

The Henry's Law Constant calculated by the programme itself is 1.02 Pa * m³/mole.

	Volume (m³)	Density (kg/m³)	org. C (g/g)	fish lipid (g/g)
Air	6.0E+9	1.185	-	-
Water	7.0E+6	1000	-	-
Soil	45000	1500	0.02	-
Sediment	21000	1300	0.05	-
Susp. sed.	35	1500	0.167	-
Fish	7	1000	-	0.05
Aerosol	0.12	1500	-	-

Result: According to the calculation, most of the substance will be distributed to air, water, soil and sediment:

	Amount (%)
Air	12.0
Water	33.9
Soil	26.8
Sediment	27.1
Susp. sed.	0.1741
Fish	0.0169
Aerosol	0.0016

Reliability: (2) valid with restrictions
Scientifically acceptable method
Flag: Critical study for SIDS endpoint
19-NOV-2004 (79)

3.4 Mode of Degradation in Actual Use

3.5 Biodegradation

Type: aerobic
Inoculum: other: no information
Contact time: 28 day(s)
Degradation: = 50 % after 28 day(s)
Result: other: not readily biodegradable

Method: OECD Guide-line 301 C "Ready Biodegradability: Modified MITI Test (I)"
Year: 1998
GLP: no data

Remark: Most likely the BOD of the ThOD was measured. It is a BOD of 50 % for two parallels indicated.
The substance is indicated as inherently biodegradable.
Test substance: Beta-ionone, indication of CAS number and purity is missing
Reliability: (4) not assignable
only a summary available, data not sufficiently documented, data confined to the above, no data on test substance and purity available
09-NOV-2004 (80)

Type: aerobic
Inoculum: other: most likely domestic sludge
Concentration: 30 mg/l related to Test substance
Contact time: 28 day(s)
Degradation: = 97 % after 28 day(s)
Result: inherently biodegradable

Method: OECD Guide-line 302 C "Inherent Biodegradability: Modified MITI Test (II)"
Year: 1989
GLP: no data

Remark: Neither kinetics nor information about the reference substance used is avialable.
Result: No inhibition of the degradation of the substrate could be observed, therfore the substance was also considered less toxic to microorganisms.
Test substance: Beta-ionone, indication of CAS number and purity is missing
Reliability: (4) not assignable
only a data compilation, no data on GLP, purity of the test substance, available information confined to the above
09-NOV-2004 (81)

Type: aerobic
Inoculum: activated sludge, domestic
Concentration: 50 mg/l related to Test substance
Degradation: = 79 % after 28 day(s)
Result: readily biodegradable
Kinetic:

1 day(s)	ca. 0 %
7 day(s)	ca. 0 %
8 day(s)	= 12.7 %
18 day(s)	= 64.9 %
28 day(s)	= 79 %

Control Subst.: Aniline
Kinetic:

1 day(s)	ca. 0 %
28 day(s)	= 90.2 %

Method: other: Manometric Respirometry Test according to EEC Directive 79-831 Annex V, Part C, 5.2
Year: 1988
GLP: no

Method: The test was carried out in a Sapromat respirometer using 10 ml of activated sludge from a domestic sewage treatment plant (750 mg dry mass/L) as inoculum resulting in a dry mass of 30 mg/L.
Remark: Method (EEC 79/831, Annex V, Part C, 5.2) is equivalent to OECD guideline 301 F.
Result: The biodegradation rate of the control substance aniline was 90.2% of ThOD within 28 days.

Biodegradation rates of beta-ionone during the 28 days test duration were as follows (CS = control substance: aniline; TS = test substance: beta-ionone, replicates 1-6; values as percentages of ThOD):

time (day)	CS	TS1	TS2	TS3	TS4	TS5	TS6
1	-6.8	-13.2	-11.8	-11.8	-12.5	-11.8	-13.2
7	56.5	-9.7	-6.3	2	-6.3	-7.5	0
8	68.9	-2.8	6.8	29.6	7.5	-7.6	42.7
18	84.6	53.1	66.2	87.6	55.8	65.5	61.3
28	90.2	60.7	84.1	104	70.3	73.8	81.3

Average values for the biodegradation of the test substance are -12.4% (day 1), -4.6% (day 7), 12.7% (day 8), 64.9% (day 18) and 79.0% (day 28).
Within the 10-day window (beginning on day 8), beta-ionone was biodegraded by more than 60%. This indicates ready biodegradation of beta-ionone.

Test substance: 1-(2,6,6-trimethyl-1-cyclohexene-1)-buten-2-one-3, beta-ionone; purity ca. 95 % (GC)

Reliability: (2) valid with restrictions
according to guideline, but non-GLP conditions

Flag: Critical study for SIDS endpoint

10-NOV-2004 (82)

Type: aerobic
Inoculum: activated sludge, domestic
Concentration: 10 mg/l related to Test substance
Contact time: 28 day(s)
Degradation: = 73 % after 28 day(s)
Result: readily biodegradable
Kinetic:
4 day(s) = 1 %
7 day(s) = 46 %
14 day(s) = 62 %
21 day(s) = 76 %
28 day(s) = 73 %
Control Subst.: Aniline
Kinetic:
4 day(s) = 63 %
7 day(s) = 88 %

Method: other: according to ISO 14593 (CO2-Evolution Test)
Year: 2000
GLP: yes

Method: According to the ISO 14593 the evolution of CO2 generated as a result of mineralisation of organic carbon from biodegradation is measured.
Result: Beta-Ionone can be considered readily biodegradable.
Mineralisation of 73 % of the theoretical carbon present was observed after 28 days, of which > 61 % occured within the defined 10 day window.
Test condition: EQUIPMENT:
- septum sealed bottles (156 mL volume)
- liquid volume per bottle: 100 mL
- test medium according to ISO 14593
- inoculum: sewage of predominantly domestic origin, filtered and sparged with CO2 free air to reduce the inorganic carbon content to < 1mg/L
- inoculum after sparging: pH adjusted to 7.0+/-0.2 and added

at 100 mL per liter of final medium
- triplicate sets for carbon analysis for each of the reference substance and test substances together with triplicate test medium blank controls
- duplicate set of abiotic controls
- test bottles sealed with butyl rubber septa and aluminium crimp caps
- test temperature: 20 +/- 2°C
- incubation: for 28 days in the dark while shaken at a nominal 150 rpm
- measurement of CO2: Dohrman DC 190 analyser

PREPARATION:
- 1.23 mg beta-Ionone was weighed an directly transferred into appropriate bottles
- aniline (reference substance) was added as 5mL aliquot of a 258 mg/L stock solution to each reference bottle
- blank control and test bottles were made up to 100 mL by using 5 mL deionised water

Test substance: beta-Ionone (CAS: 14901-07-6, undefined stereoisomerie at the buten double bond), supplied by Quest International, Ashford, Kent TN24 0LT, UK); purity: min. 95%

Reliability: (1) valid without restriction
Guideline study under GLP conditions

Flag: Critical study for SIDS endpoint

09-NOV-2004 (83)

Type: aerobic
Inoculum: other: none
Concentration: 6.28 µg/l related to Test substance
Degradation: ca. 95 % after 20 hour(s)

Method: other: biodegradation test using spiked river water
Year: 1992
GLP: no data

Method: Water from Murrumbidgee River was collected. Part of the water was stripped and analysed as a blank. The remaining water was spiked with a mixture of beta-ionone (6280 ng/l) and further volatile compounds (6-methylhept-5-en-2-one, geranyl acetone and beta-cyclocitral). It was incubated for 130 hours at room temperature (22°C) under normal light conditions. Sub-samples were taken at ca. 12 hour intervals, stripped and analysed by GC/MS.

Result: Beta-ionone was rapidly degraded in Murrumbidgee River water. After 20 hours, its concentration had declined to less than 5% (% Degradation was read from a figure).

Test substance: beta-ionone; neither data on which isomer was used nor the purity are given in the report

Reliability: (2) valid with restrictions
Scientifically acceptable publication

18-JUN-2004 (84)

3.6 BOD5, COD or BOD5/COD Ratio

Method:

C O D

Method: other: no information
Year: 1989
GLP: no data
COD: = 2912 mg/g substance
Method:
Remark: A TOC of 812 g/kg is indicated.
Reliability: (3) invalid
only a data compilation, no data on GLP, purity of the test substance, available information confined to the above
18-JUN-2004 (81)

3.7 Bioaccumulation

Species: other
BCF: = 508.3

Method: other: calculated using SRC BCFWIN v2.14
Year: 2003

Remark: Estimation based on the log Kow of 4.42 estimated using KOWWIN v.1.66 and the equation logBCF = 0.77 logKow - 0.70.
Reliability: (2) valid with restrictions
Scientifically acceptable method
21-MAR-2004 (85)

Species: other: fish
BCF: = 501

Method: other: calculated according to TGD (2003)
Year: 2004

Result: Using the equation logBCF (fish) = 0.85 * logKow - 0.70 developed by Veith et al. (1979; see TGD, Part III, 2003, p. 31) and the measured lowKow of 4.0 (see chapter 2.5), a BCF of 501 was calculated.
Reliability: (2) valid with restrictions
Scientifically acceptable method
Flag: Critical study for SIDS endpoint
24-MAR-2004 (76) (86)

3.8 Additional Remarks

AQUATIC ORGANISMS

4.1 Acute/Prolonged Toxicity to Fish

Type: flow through
Species: Pimephales promelas (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** yes
LC50: = 5.09
Limit Test: no

Method: other: according to US EPA Committee on Methods for Toxicity Tests with Aquatic Organisms (1975)
Year: 1988

Remark: The result is based on nominal values because the percent recovery for analytical verification was 103.5+/-7.9%.
Result: During the 96 h exposure to beta-ionone, mortalities were as follows:

Nominal test concentration	Cumulative mortality (%) after 24 h	48 h	72 h	96 h
Control	0	0	0	0
1.47 mg/l	0	0	0	0
2.26 mg/l	0	0	0	0
3.47 mg/l	0	0	0	0
5.34 mg/l	10	10	10	10
8.22 mg/l	100	100	100	100

Maximum test concentration causing no mortality: 3.47 mg/l (nominal) (=measured: 2.54 mg/l).
Minimum test concentration causing 100% mortality: 8.22 mg/l (nominal) (= measured: 6.98 mg/l).
The trimmed Spearman-Karber 96 h LC50-value was 5.09 mg/l (95% confidence limits: 4.75-5.44 mg/l).

Observations on fish behaviour and appearance: percentage of fish exhibiting behavioural effects (see below) including death as an effect

Nominal test concentration	% affected fish after 24 h	48 h	72 h	96 h
Control	0	0	0	0
1.47 mg/l	0	0	0	0
2.26 mg/l	0	0	0	0
3.47 mg/l	10	10	10	0
5.34 mg/l	30	30	30	25
8.22 mg/l	100	100	100	100

Affected fish lost schooling behaviour, were hypoactive and underreactive to external stimuli and had rigid musculature. They were darkly coloured and lost equilibrium prior to death.
The trimmed Spearman-Karber 96 h EC50-value was 4.71 mg/l (95% confidence limits: 4.27-5.20 mg/l).
Test condition: Age of the test fish: 29 days
Length of test fish: 20.0+/-1.9 mm
Weight of test fish: 0.122+/-0.035 g
Tank volume: 2.0 l

One test vessel per treatment
No. of fish per test vessel: 20
Loading: 1.220 g/l
Renewal of test water: 18 chamber additions/day
Temperature: 25.5+/-0.4°C
Dissolved oxygen: 6.8+/-0.3 mg/l
Hardness: 44.0+/-0.4 mg CaCO3/l
Alkalinity: 43.3+/-0.3 mg CaCO3/l
pH-value: 7.8+/-0.1

Stock solution of beta-ionone (42.3 mg/l) were prepared by blending.
Concentrations of the test substance in the test vessels were measured on days 0 (onset of test), 1, 2, 3 and 4 by gas-liquid chromatography and were as follows (concentrations are indicated in mg/l; corrected average = mean measured concentration corrected for percent recovery):

Nominal concentr.	Control	1.47 mg/l	2.26 mg/l	3.47 mg/l	5.34 mg/l	8.22 mg/l
day 0	<0.4	1.61	1.97	2.92	4.26	7.17
day 1	<0.4	1.38	1.75	2.65	4.38	7.49
day 2	<0.4	1.36	1.85	2.68	4.40	7.66
day 3	<0.4	1.59	1.69	2.49	4.06	6.88
day 4	<0.4	1.42	1.80	2.41	4.14	6.91
Average	<0.4	1.47	1.81	2.63	4.25	7.22
Corrected average	<0.39	1.42	1.75	2.54	4.10	6.98

Percent recovery for analytical method: 103.5+/-7.9%

Test substance: CAS 79-77-6, beta-ionone (Aldrich Chemical Co.), purity: 98%

Reliability: (1) valid without restriction
Guideline study. Most sensitive study available on this endpoint. Flow-through test system, concentration control analytics were performed.

Flag: Critical study for SIDS endpoint

15-AUG-2005 (87)

Type: other: no information
Species: Oncorhynchus mykiss (Fish, fresh water)
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no data
LC0: = 5
LC100: = 10

Method: other: no information
Year: 1989
GLP: no data

Remark: Rainbow trouts of 6 - 8 cm in length were used. The test was conducted at 16 +/-1°C for 48 hours.

Reliability: (4) not assignable
only a data compilation, no data which method was used and on GLP, purity of the test substance; available information confined to the above

16-NOV-2004 (88)

Type: static
Species: Leuciscus idus (Fish, fresh water)
Exposure period: 96 hour(s)

Unit: mg/l **Analytical monitoring:** no
LC50: 6.8
Limit Test: no

Method: other: German Industrial Standard DIN 38412, Part 15
Year: 1989
GLP: no

Method: The test was carried our according to the German Industrial Standard Guideline DIN 38412, Part 15 (1982), using a static exposure procedure.
Remark: According to the guideline, the corpulence factor K should be between 0.8 and 1.1 g/cm³ (equation for calculation: $K = 100*W/L^3$; W = weight in g; L = length in cm). This criterion was fulfilled.
Result: The LC50 (48 h) of the positive control chloroacetamide was 23 mg/l, which corresponds to the normal sensitivity of the fish.

During the 96 h exposure to beta-ionone, mortalities were as follows:

Nominal test concentration (mg/l)	Cumulative mortality (%) after 1 h	4 h	24 h	48 h	72 h	96 h
Control	0	0	0	0	0	0
Solvent control	0	0	0	0	0	0
1.00	0	0	0	0	0	0
2.15	0	0	0	0	0	0
4.64	0	0	0	0	0	0
10.00	80	90	90	100	100	100

Tumbling of fish was observed in the treatment with 4.64 mg/l after 4 h of exposure (but not later throughout the test). Fish in a narcotic state were observed in the highest test concentration after 1 and 24 h.

Maximum test concentration causing no mortality: 4.64 mg/l.
Minimum test concentration causing 100% mortality: 10.00 mg/l.
According to OECD 203 (1992), the LC50 (96 h) was obtained by calculating the geometric mean of these two concentrations: it was 6.81 mg/l.

All values refer to nominal concentrations of beta-ionone.
No chemical analysis of the test substance was carried out.
Test condition: Test fish: Leuciscus idus (Golden variety)
body length: 6.0 cm (5.5-7.1 cm),
body weight: 1.8 g (1.2-2.8 g),
Corpulence factor of the batch: 0.8

Housing and adaptation:
Duration of housing: ca. 1 month
Duration of adaptation: 3 days
Mortality during last 2 weeks of housing: ca. 1.4%
Mortality during adaptation: 0%
Medical treatment: twice with 0.05 mg/l malachite green chloride, once with 10 mg/l tetracycline hydrochloride
Water temperature: 20-21°C
Diet: growing feed (ad libitum); withdrawal of food 1 day before and during exposure

Test procedure:
Test water: reconstituted freshwater according to DIN 38412, Part II, 1982) that was prepared from fully demineralised tap water by addition of 294.0 mg/l CaCl2*2 H2O, 123.3 mg/l MgSO4*7 H2O, 63.0 mg/l NaHCO3 and 5.5 mg/l KCl. The test water had a total hardness of 2.5 mmol/l, an acid capacity of 0.8 mmol/l and a pH of ca. 8.0.
Photoperiod: 16:8 h (light:dark)
Temperature: 22-23°C
Aeration: continuous
Test vessels: all-glass aquaria (30 * 22 * 22 cm) filled with 10 l of test water, one test vessel per treatment
No. of fish/test vessel: 10
Loading: 1.8 g fish/l test water

Nominal test concentrations: 1.00, 2.15, 4.64 and 10 mg/l
The test substance was dissolved in acetone (1.0 g/100 ml), this solution was added to the aquaria. The highest solvent concentration was 1 ml/l of water. A solvent control with the same solvent concentration was included in the test.
No undissolved test substance was visible in the aquaria.
Positive control: chloroacetamide
Oxygen concentrations measured in all test vessels 1, 24, 48, 72 and 96 h after the beginning of the test ranged from 7.2 mg/l to 8.4 mg/l, pH-values ranged from 7.4 to 7.7 and temperature from 22°C to 23°C.

Test substance: Beta-ionone, purity: 95%
Reliability: (2) valid with restrictions
Guideline study according to national standard protocol

15-FEB-2005 (89)

4.2 Acute Toxicity to Aquatic Invertebrates

Type: static
Species: Daphnia magna (Crustacea)
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** yes
EC50: = 3.7

Method: OECD Guide-line 202
Year: 2004
GLP: yes

Remark: The 48-h LC50 value is based on measured data.
Result: The number of mobile daphnids after hours of exposure in the different test concentrations was as following:

Concentration	repl. 1		repl. 2		repl. 3		repl. 4		Sum	
[mg/l]	24	48	24	48	24	48	24	48	24	48
0	5	5	5	5	5	5	5	5	20	20
1	5	5	5	5	5	5	5	5	20	20
2	5	4	5	5	5	5	5	4	20	18
4	4	4	4	4	4	1	4	4	16	13
8	1	0	1	0	1	0	1	0	6	0
16	0	0	0	0	0	0	0	0	0	0

The EC50 (48 h) calculated using the Probit analyses (Finney, 1971) was 4.03 mg/l (confidence limits 95%:3.36 - 4.92 mg/l).

Concentration control analyses performed by HPLC indicate that initial concentration was between 90 and 92 % of nominal conentration and reduction during the test period (48 h) was below 20%.
The percent recovery was as follow:

nominal conc. [mg/l]	measured (0h; %)	measured (48h; %)
1	91.1	78.2
2	90.9	76.9
4	92.0	74.8
8	91.4	73.8
16	90.1	75.4

Therefore, the results were related to initial measured concentrations.

O2-concentrations and pH-values were measured in all test vessels. The oxygen concentrations were 8.8 - 9.1 mg/l, the pH values ranged from 7.5 to 7.8.

Test condition: A synthetic fresh water was used for culture and test purposes. Properties of this medium:
Total hardness: 2,20 - 3,20 mmol/l
Alkalinity up to pH 4.3: 0,80 - 1,00 mmol/l
Molar ratio Ca:Mg: about 4 : 1
pH value: 7,5 - 8,5
Conductivity: 550 - 650 µS/cm

After preparation the M4 medium was aerated for approximately 24 h until saturation.

Duration of the exposure: 48 hours
Test temperature: 18 - 22 °C
Test vessel: Test tubes (glass), nominal volume: 20 ml
Test volume: 10 ml
Loading (animals/ml): 0.5
Number of animals/vessel: 5
Total number of animals/conc.: 20
Number of replicates: 4
Age of the animals at the beginning of exposure: 2 - 24 h
Age of stock animals: 2 - 4 weeks
Illumination: Artificial light, 16 light : 8 h dark
Test parameter: Swimming ability of the test animals after 0, 24 and 48 h
Feeding: none

The test substance was tested in the range of concentrations between 16 and 1 mg/l. The dilution factor was 2.
Concentration control analysis were performed after 24h and 48h.

Test substance: beta-Ionone R, CAS: 79-77-6, purity: 97.8% (area)

Reliability: (1) valid without restriction
Guideline study under GLP conditions, analytics included

Flag: Critical study for SIDS endpoint

15-AUG-2005 (90)

Type: static

Species: Daphnia magna (Crustacea)

Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no
EC50: = 1.1
Limit Test: no

Method: other: according to DIN 38412 Part 11
Year: 1991
GLP: no

Remark: The test was performed according to DIN 38412, part 11 using daphnids from a in-house breeding stock using a static exposure procedure. As solubilising agent Cremophor was used (concentration from 0.018 mg/l to 3.2 mg/l).
After preparing a stock solution using Cremophor the following nominal concentrations were tested: 0.18, 0.32, 0.58, 1.0, 1.8, 3.2, 5.8, 10, 18 und 32 mg/l. For each concentration and for the control and solvent control 4 replicates were used.
O2-concentrations and pH-values were measured in all test vessels. The oxygen concentrations were 7.6 - 7.7 mg/l, the pH values ranged from 7.80 to 7.87.
No chemical analysis of the test substance was carried out.

Result: The EC50 values calculated according to Spearman-Karber-method were 4.5 mg/l (24h) and 1.0 mg/l (48h). Using Probit analysis (ToxRat V2.08), an EC50 (24h) of 4.73 mg/L (confidence interval: 3.36 - 6.67 mg/l) and an EC50 (48h) of 1.09 mg/l (confidence interval: 0.83 - 1.44 mg/l) were calculated.

Test substance: Beta-ionone, CAS: 79-77-6, purity: 97.2 %, contains alpha-ionone and 1.4 % other impurities

Reliability: (2) valid with restrictions
Guideline study according to national standard protocol

15-FEB-2005 (91) (92)

4.3 Toxicity to Aquatic Plants e.g. Algae

Species: Scenedesmus subspicatus (Algae)
Endpoint: growth rate
Exposure period: 72 hour(s)
Unit: mg/l **Analytical monitoring:** no
EC10: = 7.1
EC50: = 22.2
Limit Test: no

Method: other: algal growth inhibition test according to German Industrial Standard DIN 38412, part 9
Year: 1989
GLP: no

Method: The test closely followed the German Industrial Standard Guideline DIN 38412, part 9. The test duration was 96 h.

Remark: The concentration of the solvent Cremophor ranged from 0.25 mg/l in the lowest to 5.0 mg/l in the highest test concentration. Cell density in the solvent control was 12% lower than in the control without solvent.

Result: Neither growth promotion induced by the test substance nor autofluorescence were observed.

During the 96 h exposure to beta-ionone, cell densities (number of cells * 1000/ml) were as follows (average of the 4 replicates):

Nominal test concentration	Cell density (n * 1000/ml) after 0 h	24 h	48 h	72 h	96 h
Control	12	25	62	205	567
Solvent control	12	24	56	197	496
2.5 mg/l	12	26	52	159	439
5.0 mg/l	12	25	54	167	449
10.0 mg/l	13	26	52	151	391
25.0 mg/l	12	23	33	39	46
50.0 mg/l	12	20	21	21	20

Growth rates and percent inhibition of biomass after 72 and 96 h were:

Nominal test concentration	Growth rate 72 h	96 h	% Biomass inhibition 72 h	96 h
Control	0.95	0.96	-	-
Solvent control	0.93	0.93	-	-
2.5 mg/l	0.86	0.90	12.2	15.7
5.0 mg/l	0.88	0.91	8.8	12.4
10.0 mg/l	0.82	0.85	15.4	22.1
25.0 mg/l	0.39	0.34	61.6	83.1
50.0 mg/l	0.19	0.13	79.2	92.5

Based on calculations according to Tallarida and Jacob, (1979), the EC-values (with 95% confidence intervals) for the endpoint growth rate were:

EμC10 (72 h) = 4.94 mg/l (1.98-12.38 mg/l)
EμC50 (72 h) = 22.91 mg/l (9.35-56.12 mg/l)
EμC10 (96 h) = 8.57 mg/l (5.29-13.87 mg/l)
EμC50 (96 h) = 21.92 mg/l (14.38-33.42 mg/l)
The EC-values for the endpoint biomass inhibition were:
EBC10 (72 h) = 3.80 mg/l (1.25-11.63 mg/l)
EBC50 (72 h) = 20.93 mg/l (8.25-53.12 mg/l)
EBC10 (96 h) = 3.15 mg/l (0.96-10.32 mg/l)
EBC50 (96 h) = 12.18 mg/l (5.58-26.58 mg/l)

Using Probit analysis (ToxRat V. 2.08), the EC-values (with 95% confidence intervals) for the endpoint growth rate were:
EμC10 (72 h) = 7.10 mg/l (1.76-11.52 mg/l)
EμC50 (72 h) = 22.15 mg/l (14.96-32.54 mg/l)
EμC10 (96 h) = 8.46 mg/l (3.46-12.16 mg/l)
EμC50 (96 h) = 20.51 mg/l (15.32-26.57 mg/l)
The EC-values for the endpoint biomass inhibition were:
EBC10 (72 h) = 6.19 mg/l (0.64-11.11 mg/l)
EBC50 (72 h) = 21.15 mg/l (12.29-37.04 mg/l)
EBC10 (96 h) = 6.22 mg/l (0.00-10.92 mg/l)
EBC50 (96 h) = 14.75 mg/l (4.60-60.76 mg/l)

All values refer to nominal concentrations of beta-ionone.
No chemical analysis of the test substance was carried out.

Test condition: Test organism: Scenedesmus subspicatus, Chodat, obtained from the Collection of Algal Cultures, University of Göttingen (SAG 86.81).

Test procedure:
According to the SOP A38412 L9. The following nominal concentrations were tested: 2.5, 5.0, 10.0, 25.0 und 50.0 mg/l. For each concentration and for the controls, 4

replicates were carried out.
A stock solution of beta-ionone was prepared using the solvent Cremophor and ultrasonication. The highest solvent concentration was 5.0 mg/l of water. A solvent control with the same solvent concentration was included in the test. Algal biomass was determined with a fluorimeter at the onset of the test and 24, 48, 72 and 96 h later.

The pH-values at the beginning (0 h) and at end of the test (96 h) and temperatures were:

Nominal test concentration	pH (0 h)	pH (96 h)	T (°C)
Control	7.73	7.99	21.3
Solvent control	7.78	7.72	21.3
2.5 mg/l	7.77	8.74	21.3
5.0 mg/l	7.76	8.94	21.3
10.0 mg/l	7.78	7.56	21.3
25.0 mg/l	7.77	7.53	21.3
50.0 mg/l	7.77	7.54	21.3

Test substance: Beta-ionone, CAS: 79-77-6, purity: 97.2 %, contains alpha-ionone and 1.4 % other impurities
Reliability: (2) valid with restrictions
National standard specification
Flag: Critical study for SIDS endpoint
15-FEB-2005 (93) (94) (95)

Species: Chlorella pyrenoidosa (Algae)
Endpoint: other: growth
Unit: **Analytical monitoring:** no
Limit Test: no

Method: other: paper disk-agar plate method
Year: 2001

Method: Various volatile organic compounds produced by cyanobacteria and algae in freshwater lakes were tested for their ability to inhibit growth of the green alga Chlorella pyrenoidosa using the paper disk-agar plate method.
Result: Growth of Chlorella was inhibited by a beta-ionone concentration of 5 mg/l (formation of a 4 mm inhibition zone). A concentration of 10 mg/l inhibited growth of Chlorella over the entire plate.
Reliability: (2) valid with restrictions
Scientifically acceptable method
17-NOV-2004 (96)

4.4 Toxicity to Microorganisms e.g. Bacteria

Type: aquatic
Species: Pseudomonas putida (Bacteria)
Exposure period: 30 minute(s)
Unit: mg/l **Analytical monitoring:** no
EC10: = 8300
EC50: > 10000
EC90 : > 10000

Method: other: oxygen consumption inhibition test according to Robra
Year: 1988

GLP: no

Method: The test was performed according Robra, 1976 (GWF-Wasser/Abwasser 117, 80-86) at a temperature of 25°C using a 48 h old bacterial suspension. The stock solution (1000 mg beta-ionone/l) was prepared using 100 mg/l of Tween 80. The test concentrations (1250, 2500, 5000 and 10000 mg/l; two replicates for each concentration) were prepared under stirring. A blank containing Tween was used.

Remark: The tested concentrations of beta-ionone had a relatively small effect on respiration (2-13%) and there was no clear concentration-effect relationship.

Result:

Concentration	pH	Oxygen consumption (1)	(2)	Mean	Mean (%)
Blank	7.14	2.35	2.3	2.33	93
Blank + Tween	7.01	2.45	2.55	2.5	100
1250 mg/l	6.98	2.4	2.4	2.4	96
2500 mg/l	6.97	2.4	2.2	2.3	92
5000 mg/l	6.96	2.45	2.45	2.45	98
10000 mg/l	6.99	2.0	2.35	2.18	87

The mean oxygen consumption rates (%) refer to the blank containing Tween.

Test substance: 1-(2,6,6-trimethyl-1-cyclohexene-1)-buten-2-one-3, beta-ionone; purity ca. 95 % (GC)

Reliability: (2) valid with restrictions
Scientifically acceptable method

10-NOV-2004 (97)

Type: other: aerobic
Species: activated sludge
Exposure period: 3 hour(s)
Unit: mg/l **Analytical monitoring:** no
EC50: = 100 - 120

Method: OECD Guide-line 209 "Activated Sludge, Respiration Inhibition Test"
Year: 1990
GLP: no
Test substance: other TS: Beta-Ionone CAS: 14901-07-6; purity: 98.4 % (GLC)

Result: The following respiration rates were measured (C = control; S = sample; R = reference substance):

	Respiration rate	Inhibition [%]	Concentration [mg/l]
C1	0.500	0	0
C2	0.513	0	0
S1	0.435	14	25
S2	0.460	9	25
S3	0.307	39	75
S4	0.278	45	75
S5	0.112	78	225
S6	0.103	80	225
S7	0.084	83	500
S8	0.097	81	500
R1	0.143	72	40

An EC 50 value for beta - Ionone of 100 - 200 mg/L can be derived.

Test condition: EQUIPMENT:

Vessels:	500 mL cylinders
Water:	drinking water
Air supply:	clean, oil-free compressed air
Measuring apparatus:	BOD flask
Test substance:	freshly ethanolic solutions
Temperature:	20 +/- 2°C
Activated sludge:	from a biological WWTP (near to Geneva)
Contact time:	3 h
reference substance:	3,5-dichlorophenol (purity: min. 99%)

TEST CONCENTRATIONS:
0, 25, 75, 225 and 500 mg/L beta-ionone were used for this study.

Reliability: (2) valid with restrictions
guideline study, non GLP, but best documented

Flag: Critical study for SIDS endpoint

15-AUG-2005 (98)

Type: other: aerobic
Species: activated sludge, domestic
Exposure period: 30 minute(s)
Unit: mg/l **Analytical monitoring:** no
EC50: ca. 1000
EC20 : ca. 53

Method: other: ISO/DIS 8192, Part B: Water quality - Test for inhibition of oxygen consumption of activated sludge. Draft, 1984
Year: 1988
GLP: no

Method: Test mixtures (total volume: 250 ml) containing synthetic medium, inoculum (activated sludge) and three concentrations of beta-ionone (50, 100 and 1000 mg/l) were incubated for 30 min at 20-25°C. Then, the respiration rate was measured.

Result:

Concentration of beta-ionone	Respiration rate (mg/l*h)	Inhibition of respiration (%)
60 mg/l	20	17
100 mg/l	15	37
1000 mg/l	13	46

The percentages inhibition of respiration refer to the mean respiration rate of the inoculum blank that was 24 mg/l*h. A disturbance of the biodegradation processes in activated sludge is possible.

Test substance: 1-(2,6,6-trimethyl-1-cyclohexene-1)-buten-2-one-3, beta-ionone; purity ca. 95 % (GC)

Reliability: (2) valid with restrictions
Scientifically acceptable method in which a municipal inoculum is used.

Flag: Critical study for SIDS endpoint

10-NOV-2004 (99)

Type: other: aerobic
Species: activated sludge, industrial
Exposure period: 30 minute(s)

Unit: mg/l **Analytical monitoring:** no
EC50: = 1000
EC20 : ca. 65

Method: other: ISO/DIS 8192, Part B: Water quality - Test for inhibition of oxygen consumption of activated sludge. Draft, 1984
Year: 1988
GLP: no

Method: Test mixtures (total volume: 250 ml) containing synthetic medium, inoculum (activated sludge) and three concentrations of beta-ionone (50, 100 and 1000 mg/l) were incubated for 30 min at 20-25°C. Then, the respiration rate was measured.
Result:

Concentration of beta-ionone	Respiration rate (mg/l*h)	Inhibition of respiration (%)
50 mg/l	22	15
100 mg/l	19	27
1000 mg/l	13	50

The percentages inhibition of respiration refer to the mean respiration rate of the inoculum blank that was 26 mg/l*h. A disturbance of the biodegradation processes in activated sludge is possible.
Test substance: 1-(2,6,6-trimethyl-1-cyclohexene-1)-buten-2-one-3, beta-ionone, no CAS number and purity is indicated
Reliability: (2) valid with restrictions
Scientifically acceptable method in which a mixed inoculum is used.
16-AUG-2004 (99)

Type: other
Species: other bacteria: Colletotrichum musae
Unit: **Analytical monitoring:** no

Method: other: determination of MIC in Petri dishes
Year: 2002
GLP: no

Method: The minimum inhibitory concentrations (MICs) of several volatile organic compounds (VOCs) to stop the growth of the microorganisms as a vapour were determined as follows. Agar plugs containing the microorganisms were transferred to the centre of Petri dishes with new agar, while filter disks containing various concentrations of the volatile organic compounds were attached to the inner surface of the lid of the Petri dish.
Result: Beta-ionone was germistatic to Colletotrichum musae (MIC: 4.65 mmol/dish). It had no germicidal or germistatic effect on Rhizopus stolonifer, Penicillium digitatum, Colletotrichum musae, Erwinia carotovora and Pseudomonas aeruginosa.
Reliability: (2) valid with restrictions
Scientifically acceptable method
16-FEB-2004 (100)

Type: other
Species: other bacteria: Escherichia coli, E. coli O157:H7, Salmonella typhimurium, Listeria monocytogenes and Vibrio vulnificus
Unit: **Analytical monitoring:** no

Method: other: paper disk method
Year: 1995

Method: Antibacterial activity of essential oil constituents against Escherichia coli, E. coli O157:H7, Salmonella typhimurium, Listeria monocytogenes and Vibrio vulnificus was tested using a zone of inhibition assay. Bacteria were spread on agar plates. Sterilised filter paper disk were arranged on the plates and 10 µl aliquots of a solution containing 5, 10, 15 or 20% of the test compound in sterile distilled water with 1% Tween 20 was added to each paper disk. Following 24 hours of incubation at 35°C, diameters of inhibition zones were measured.
Result: At the tested concentrations, beta-ionone did not show any inhibition against E. coli, E. coli O157:H7 and S. typhimurium. It showed a weak but dose-related antibacterial activity against V. vulnificus, while inhibition of L. monocytogenes was observed but not dose-related.
Reliability: (2) valid with restrictions
Scientifically acceptable method
16-FEB-2004 (101)

Type: other
Species: other bacteria: e.g. Bacillus subtilis, and other fungi
Unit: **Analytical monitoring:** no

Method: other: determination of MIC by the broth dilution method
Year: 1992
GLP: no

Method: The antimicrobial activity of volatile compounds against various bacteria and fungi was examined by the broth dilution method. Solution of the test compound was added to 2-day-old cultures of the microorganisms. After 2-5 days of incubation, growth of the microorganisms was checked. Minimal inhibitory concentrations (MICs) were measured by twofold serial broth dilution.
Result: Antimicrobial activity of beta-ionone against the microorganisms was as follows:

	MIC (µg/ml)
Bacillus subtilis	100
Brevibacterium ammoniagenes	100
Staphylococcus aureus	200
Streptococcus mutans	100
Propionibacterium acnes	25
Pseudomonas aeruginosa	>800
Enterobacter aerogenes	>800
Escherichia coli	>800
Saccharomyces cerevisiae	>800
Candida utilis	400
Pityrosporum ovale	>800
Penicillium chrysogenum	400
Trichophyton mentagrophytes	50

Test substance: Beta-ionone
Reliability: (2) valid with restrictions
Scientifically acceptable method
16-FEB-2004 (102)

Type: other

Species: other fungi: Aspergillus parasiticus
Unit: **Analytical monitoring:** no

Method: other
Year: 1986
GLP: no

Method: The inhibitory effect of beta-ionone on growth and sporulation of Aspergillus parasiticus was investigated by (1) direct application of beta-ionone to the agar surface and (2) by exposure via the volatile phase. Suspension of A. parasiticus spores was inoculated onto mycological agar plates and different amounts of beta-ionone were pipetted (1) onto the agar plates (5-50 µl) and (2) onto the inside of the cover lids (5-100 µl). The plates were sealed and incubated for 2 weeks at 28°C.
Result: Beta-ionone (5, 25 and 50 µl) placed on the agar surface or applied via the volatile phase (1-100 µl) resulted in restricted growth of A. parasiticus colonies and inhibited (application on agar) or reduced (volatile phase) sporulation. When mycelia were transferred to fresh agar, they resumed growth, i.e. the effect of beta-ionone was temporary.
Reliability: (2) valid with restrictions
Scientifically acceptable method
16-FEB-2004 (103)

Type: other
Species: other fungi: Peronospora tabacina
Unit: **Analytical monitoring:** no

Method: other: determination of MIC in Petri dishes
Year: 1987
GLP: no

Method: Germination, appressorial formation, vesicle production and sporulation of Peronospora tabacina on leaf surfaces was studies using an in situ staining technique.
Result: Beta-ionone inhibited germination and formation of the appressiorium of the fungus Peronospora tabacina.
Reliability: (2) valid with restrictions
Scientifically acceptable method
11-FEB-2004 (104)

4.5 Chronic Toxicity to Aquatic Organisms

4.5.1 Chronic Toxicity to Fish

4.5.2 Chronic Toxicity to Aquatic Invertebrates

TERRESTRIAL ORGANISMS

4.6.1 Toxicity to Sediment Dwelling Organisms

4.6.2 Toxicity to Terrestrial Plants

4.6.3 Toxicity to Soil Dwelling Organisms

4.6.4 Toxicity to other Non-Mamm. Terrestrial Species

Species: other: avian (red-winged blackbird; Agelaius phoeniceus)
Endpoint: mortality
Expos. period: 18 hour(s)
Unit: mg/kg bw
LD50 : > 562

Method: other: Acute oral toxicity
Year: 1972
GLP: no data

Remark: Estimated LD50 are based on food consumption data.
Test condition: -birds were preconditioned to captivity for 2 to 6 weeks and dosed by gavage with solutions or suspensions of the test chemical in propylene glycole
- other oral dosing methods were occasionally used like pellets or gelatine capsules
Reliability: (2) valid with restrictions
basic data are given and are acceptable, even if no informations about the number of test organisms, the way and preparation of dosed gavages and other important test parameters for the corresponding test substances are available
Flag: Critical study for SIDS endpoint
18-JUN-2004 (105)

4.7 Biological Effects Monitoring

4.8 Biotransformation and Kinetics

Type: other: fungi (Aspergillus niger)

Method: Conical flasks: Conical flasks were filled with culture medium (sucrose, yeast, salt solutions), inoculated with Aspergillus niger (strains IFO 8541, ATCC 9142, ATCC 11394 and NCIM 612) and placed on a rotating table at 27°C. After 50-150 hours, 1 g/l of beta-ionone was added. Further beta-ionone was added when the beta-ionone concentrations had decreased to values close to zero.
Bioreactor: A thermostated, aerated, stirred bioreactor in a fed-batch mode of operation (sequential addition of beta-ionone) was used. The reactor was filled with sucrose and yeast solution enriched with calcium chloride and sterilised. Spores of A. niger (IFO 8541) were entrapped in calcium alginate beads and salt solutions were added.
Chemical analysis: Beta-ionone and reaction products were extracted with diethyl ether and analysed by GC.
Result: Biotransformation products obtained in the conical flask-experiments were:

A. niger strain Reaction products

	IFO 8541 2- and 4-oxo-beta-ionone ATCC 9142 2- and 4-hydroxy-beta-ionone NCIM 612 2- or 4-oxo-beta-ionone 2-hydroxy-beta-ionone ATCC 11394 numerous unidentified substances In the bioreactor experiment with A. niger IFO 8541, nearly all beta-ionone was transformed. Biotransformation products were 4-hydroxy-beta-ionone, 2-hydroxy-beta-ionone and 4-oxo-beta-ionone.
Reliability:	(2) valid with restrictions Scientifically acceptable method
16-FEB-2004	(106)
Type:	other: fungi (Aspergillus niger)
Remark:	Main products of biotransformation of beta-ionone by Aspergillus niger (strain IFO 8541): 4-hydroxy-beta-ionone, 4-oxo-beta-ionone, 4-hydroxy-5,6-epoxy-beta-ionone, 2-hydroxy-beta-ionone, 2-oxo-beta-ionone, 4-hexyl-2,5-dihydro-2,5-dioxo-3-furanacetic acid. Suggested transformation pathways of beta-ionone are enantioselective hydroxylation at C-2 and C-4 followed by further oxidation and/or dehydration or acetylation.
Reliability:	(2) valid with restrictions Scientifically acceptable review
16-FEB-2004	(107)
Type:	other: fungi (Aspergillus niger)
Method:	Abiotic system: Stability of beta-ionone in an abiotic system was studied using an aerated, stirred bioreactor that was filled with distilled water containing 0.96-1.92 g/l of beta-ionone. Incubation was carried out at 27°C, the solution was aerated and stirred. Biotic system: An aerated, stirred bioreactor was used. The reactor was filled with sucrose and yeast solution and sterilised. Spores of A. niger (IFO 8541) were entrapped in calcium alginate beads and salt solutions were added. Incubation was carried out at 27°C with aeration using air free of CO2. After 28 hours of fungal growth, 0.92 g/l of beta-ionone were added. Chemical analysis: Beta-ionone and reaction products were extracted with diethyl ether and analysed by GC.
Result:	In the abiotic system, beta-ionone concentration gradually decreased and about 20 reaction products were observed. Most of the peaks corresponded to compounds not registered in the mass spectry databanks that were consulted (Wiley-NBS and NIST-EPA-MSCD). Three products were clearly identified: 5,6-epoxy-5,6-dihydro-beta-ionone (main product), dihydroactinidiolide, 4-oxo-beta-ionone. In the biotic system, the transformation products were: 4-hydroxy-beta-ionone (main product), 2-hydroxy-beta-ionone, 4-hexyl-2,5-dihydro-2,5-dioxo-3-furanacetic acid,

4-oxo-beta-ionone,
4-hydroxy-5,6-epoxy-beta-ionone,
2-oxo-beta-ionone.

Test substance: Beta-ionone, 95% purity

Reliability: (2) valid with restrictions

16-FEB-2004 (108)

Type: other: fungi (Aspergillus niger)

Method: A bioreactor was filled with sucrose and yeast solution and sterilised. Spores of A. niger (IFO 8541) that were entrapped in calcium alginate beads and salt solutions were added. Incubation was carried out at 27°C with aeration using air free of CO2. After 26-120 hours of fungal growth, 0.56-0.96 g/l of beta-ionone were added. Further beta-ionone was added periodically (usually every two days).
Beta-ionone and reaction products were extracted with diethyl ether and analysed by GC.

Result: Biotransformation products were:
4-oxo-beta-ionone,
2-hydroxy-beta-ionone,
4-hydroxy-beta-ionone,
2-oxo-beta-ionone,
4-hydroxy-5,6-epoxy-beta-ionone,
4-hexyl-2,5-dihydro-2,5-dioxo-3-furanacetic acid.

The major biological metabolites were more soluble and less volatile than beta-ionone. Estimated log Kow (predicted with ACE/log P program, Ilab package), vapour pressures and water solubilities at 25°C (estimated according to Meylan et al., 1996) were:

Compound	log Kow	vapour pressure	water solubility
hydroxy-beta-ionone	1.95	0.000034	39 mol/l
oxo-beta-ionone	1.41	0.000891	49 mol/l
4-hydroxy-5,6-epoxy -beta-ionone	1.07	0.000021	243 mol/l

Reliability: (2) valid with restrictions
Scientifically acceptable method

16-FEB-2004 (109) (110)

4.9 Additional Remarks

5.0 Toxicokinetics, Metabolism and Distribution

In Vitro/in vivo: In vivo
Type: Metabolism
Species: rabbit
No. of animals, males: 1
Doses, males: about 1000 mg/kg bw, daily for 7 days, approximate total dose: 23 g
Vehicle: other: aqueous suspension with gum arabic
Route of administration: gavage
Exposure time: 7 day(s)

Method: other
Year: 1970
GLP: no
Test substance: other TS

Result: The following compounds were identified: unchanged beta-Ionone, 3-oxo-beta-Ionone, 3-oxo-beta-Ionol, dihydro-3-oxo-beta-Ionol, 3-hydroxy-beta-Ionol and the glucuronides of 3-oxo-beta-Ionol and dihydro-3-oxo-beta-Ionol.

Excretion products were isolated as 2,4-dinitrophenylhydrazone derivatives (beta-Ionone, 3-oxo-beta-Ionone, 3-oxo-beta-ionol and dihydro-3-oxo-beta-Ionol) and as p-nitrobenzoate derivatives (3-oxo-beta-Ionol, dihydro-3-oxo-beta-Ionol and 3-hydroxy-beta-Ionol), which were characterized and identified by comparison with the synthetic authentic compounds. The glucuronides of 3-oxo-beta-Ionol and dihydro-3-oxo-beta-Ionol were detected in the urine. The latter compound was isolated as free glucuronide, sodium salt and 2,4-dinitrophenylhydrazone.

Test condition: ADMINISTRATION
The test material, in aqueous suspension with gum arabic, was given by stomach tube to a male albino rabbit (approximately 1.0 g/kg bw; approximate total dose of 23 g) daily for 7 days. Urine was collected daily during dosing and for 4 days after last dose.

EXTRACTION
The urine was adjusted to pH 6.0 and extracted with ether. Free metabolites and their derivatives were separated by TLC and identified by UV and IR spectra.

Test substance: beta-Ionone; no further data on purity provided
Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, well documented and acceptable for assessment
Flag: Critical study for SIDS endpoint
22-AUG-2005 (111)

In Vitro/in vivo: In vivo
Type: Metabolism
Species: rabbit
Vehicle: no data
Route of administration: gavage
Exposure time: 18 day(s)

Method: other
Year: 1950
GLP: no
Test substance: other TS

Remark: The urinary metabolites of beta-Ionone after oral administration to rabbits were studied.
Result: After feeding the rabbits with beta-Ionone 4-oxo-beta-Ionone and 4-oxo-beta-Ionole were identified as metabolites in the urine of the animals.
Test condition: 100 g beta-Ionone was fed to 2 rabbits for a period of 18 days (daily dose about 2.8 g/animal). The urine was collected. The metabolites were extracted from the urine, separated by chromatography and identified by chemical reaction with phenyl semicarbazide and 2,4-dinitrophenyl hydrazine.
Test substance: beta-Ionone, no further data on purity
Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, well documented and acceptable for assessment
Flag: Critical study for SIDS endpoint
04-JAN-2005 (112)

5.1 Acute Toxicity

5.1.1 Acute Oral Toxicity

Type: LD50
Species: rat
Strain: Osborne-Mendel
Sex: male/female
No. of Animals: 10
Vehicle: other: undiluted substance was applied
Doses: no individual doses mentioned
Value: = 4590 mg/kg bw

Method: other
Year: 1964
GLP: no
Test substance: other TS

Result: The LD50 was reported to be 4590 mg/kg bw (95% confidence interval: 3880 - 5400 mg/kg bw). The times of death were 4 hrs to 4 days. As signs of clinical toxicity, depression and tremors were mentioned.
Test condition: TEST ORGANISMS AND ADMINISTRATION
Groups of 10 young adult Osborne-Mendel rats (evenly divided by sex) were fasted for approximately 18 hrs prior treatment. Animals had access to water at all times and the food was replaced in cages as soon as animals received their respective doses. All doses were given by intubation. No further details.

EXAMINATIONS
All animals were maintained under close observation for recording toxic signs and time of death. Such observation was continued until animals appeared normal and showed weight gain. The usual observation period was 2 weeks. LD50s were computed by the method of Litchfield and Wilcoxon (1949).

Test substance: 60 % alpha-Ionone and 40 % beta-Ionone, no further data on purity
Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, well documented and acceptable for assessment
Flag: Critical study for SIDS endpoint
25-APR-2005 (113) (114)

Type: LD50
Species: mouse
Strain: no data
Sex: no data
Vehicle: no data
Doses: no data
Value: = 5700 mg/kg bw

Method: other: no further information
Year: 1979
GLP: no data
Test substance: other TS

Test substance: Dihydro-beta-Ionone (CAS-no. 17283-81-7), purity 99%
Reliability: (4) not assignable
Secondary literature
09-NOV-2004 (115)

5.1.2 Acute Inhalation Toxicity

5.1.3 Acute Dermal Toxicity

5.1.4 Acute Toxicity, other Routes

Type: LD50
Species: mouse
Strain: no data
Sex: no data
Vehicle: no data
Doses: no data
Route of admin.: i.p.
Value: = 2277 mg/kg bw

Method: other
Year: 1963
GLP: no
Test substance: other TS

Test condition: Albino mice weighing 15-18 g were used. Test substance were applied in 1 ml of oil. Observation time 7 days.
Test substance: Ionone; no further specification is provided.
Reliability: (4) not assignable
Documentation insufficient for assessment
15-JUN-2004 (116) (117)

Type: LD50
Species: mouse
Strain: no data
Sex: no data

Vehicle: no data
Doses: no data
Route of admin.: i.p.
Value: = 1500 mg/kg bw

Method: other: no further information
Year: 1979
GLP: no
Test substance: other TS

Test substance: Dihydro-beta-Ionone (CAS-no. 17283-81-7), purity 99%
Reliability: (4) not assignable
Secondary literature
15-NOV-2004 (115)

Type: LD50
Species: mouse
Strain: other: albino mice
Sex: male
No. of Animals: 10
Vehicle: other: sesame oil
Doses: no data
Route of admin.: s.c.
Value: = 2605 mg/kg bw

Method: other
Year: 1957
GLP: no
Test substance: other TS

Result: LD50: 2605 mg/kg bw (range: 2180 - 3857 mg/kg bw)
Test condition: TEST ORGANISMS
Per dose group 10 male mice with a weight of 18-25 g, no control animals were included

ADMINISTRATION
The substance was applied with a geometric progression of 1.1.

EXAMINATIONS
Lethal doses: Results were evaluated according to Lichtfield and Wilcoxon (1948).
Central stimulating effects (convulsions) were also studied (see chapter 5.11).
Test substance: Mixture of alpha-Ionone and beta-Ionone; no further data
Reliability: (3) invalid
Unsuitable test system
15-JUN-2004 (118)

5.2 Corrosiveness and Irritation

5.2.1 Skin Irritation

Species: rabbit
Concentration: undiluted
Exposure: Semiocclusive
Exposure Time: 4 hour(s)
No. of Animals: 3
PDII: 0
Result: not irritating

EC classificat.: not irritating

Method: OECD Guide-line 404 "Acute Dermal Irritation/Corrosion"
Year: 1992
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: Methods applied:
- European Economic Community (EEC), EEC Directive 84/449, Methods for the determination of toxicity, publication no. L 251, B.4: " Acute toxicity - skin irritation", September 19, 1984
- Organization for Economic Co-operation and Development (OECD), OECD Guidelines for Testing of Chemicals, Guideline No. 404: " Acute dermal irritation / corrosion"; adopted May 12, 1981

Result: LOCAL EFFECTS
Application of test substance for 4 hrs did not lead to any skin findings.
Scores for erythema and edema: 0.0 at all readings (4, 24, 48 and 72 hrs).

SYSTEMIC TOXICITY
No mortality occurred. There were no signs of clinical toxicity from the dermal exposure.

Test condition: TEST ANIMALS
Strain: White Vienna rabbits
Sex: 1 male, 2 females
Source: M. Gaukler, Offenbach, Germany
Weight at study initiation (mean): 2.91 kg (males), 2.34 kg (females)

ADMINISTRATION/EXPOSURE
Preparation of test substance: test substance was used as delivered
Area of exposure: 2.5 cm x 2.5 cm, upper third of the back of the animals
Vehicle: not used
Total volume applied: cotton pad (size: 2.5 cm x 2.5 cm) was saturated with the test substance (0.5 ml), patches were secured in position with a porous dressing.
Exposure time: 4 hrs (semiocclusive)
Removal of test substance: after exposure substance remnants were removed with Lutrol (polyethylenglykol) and Lutrol/water dilution (1/1).
Readings: 30 - 60 minutes after removal of the test patches and 24, 48 and 72 hrs after beginning of the application
Observation period: 72 hrs
Negative controls: untreated sites of the same animal

Test substance: beta-Ionone, purity 96.9 area %
Conclusion: Semiocclusive application of undiluted beta-Ionone for 4 hrs to rabbit skin did not lead to any signs of irritation.
Reliability: (1) valid without restriction
GLP guideline study
Flag: Critical study for SIDS endpoint
15-JUN-2004 (119)

Species: rabbit
Concentration: undiluted
Exposure: Occlusive

Exposure Time: 24 hour(s)
No. of Animals: 3
Vehicle: other: none
Result: slightly irritating

Method: other: comparable to Draize method
Year: 1967
GLP: no
Test substance: other TS

Result: Primary skin irritation
(scores for individual animals)

	24 hrs		48 hrs	
	abraded	unabraded	abraded	unabraded
Erythema	2, 1, 2	2, 1, 2	2, 2, 2	2, 2, 2
Edema	1, 1, 1	1, 1, 1	1, 0, 0	1, 0, 0

Test condition: The compound was applied to abraded and unabraded rabbit skin at full strength. 3 rabbits were used. Untreated intact and abraded skin served as controls on the same rabbits. The treated and untreated areas were observed after 24 and 48 hrs for signs of irritation. Assessment of findings according to Draize scores.

Scale used for scoring:
0 = normal, 1 = very slight, 2 = well defined,
3 = moderate, 4 = severe
Test substance: beta-Ionone, no further data on purity
Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, well documented and acceptable for assessment
15-NOV-2004 (120)

Species: rabbit
Concentration: 5 %
Exposure: Occlusive
Exposure Time: 24 hour(s)
No. of Animals: 3
Vehicle: other: diethylphthalate
Result: slightly irritating

Method: other: comparable to Draize method
Year: 1967
GLP: no
Test substance: other TS

Result: Primary skin irritation
(scores for individual animals)

	24 hrs		48 hrs	
	abraded	unabraded	abraded	unabraded
Erythema	2, 1, 0	2, 1, 0	0, 0, 0	0, 0, 0
Edema	1, 1, 0	1, 1, 0	0, 0, 0	0, 0, 0

Test condition: The compound was applied to abraded and unabraded rabbit skin at 5% concentration in diethylphthalate. 3 rabbits were used. Untreated intact and abraded skin served as controls on the same rabbits. The treated and untreated areas were observed after 24 and 48 or 72 hrs for signs of irritation. Assessment of findings according to Draize scores.

Scale used for scoring:
0 = normal, 1 = very slight, 2 = well defined,
3 = moderate, 4 = severe

Test substance: beta-Ionone, no further data on purity
Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, well documented and acceptable for assessment

15-NOV-2004 (120)

Species: guinea pig
Concentration: 50 %
Exposure: Open
Exposure Time: 70 minute(s)
No. of Animals: 5
Vehicle: other: acetone
PDII: 0
Result: not irritating

Method: other: Morikawa F et al. (1974). Techniques for evaluation of phototoxicity and photoallergy in laboratory animals and man, Sunlight and Man, University of Tokyo Press, Japan, 529-557
Year: 1992
GLP: no data
Test substance: other TS

Method: Acute dermal irritation and phototoxicity test by topical application.
Result: No evidence of erythema and edema was noted after treatment with beta-ionone at a maximum concentration of 50% (w/v) in acetone with or without UV-A irradiation. The primary irritation index was 0.0 for all test groups.
Test condition: TEST ANIMALS
5 female albino Hartley Guinea pigs were used purchased from Japan SLC, Inc.
ADMINISTRATION/EXPOSURE
Hair on the back of the animal was cut with an electric hair clipper and an electric shaver. 4 hours after depilation, the test agent dissolved in acetone was applied on a circle of 1.5 cm in diameter in the depilated area on both sides of the animal. A total of 8 such applications were made (one spot each of a 50%, 30%, 10% and 5% solution in acetone on the right and the left side).

Immediately after application, one side was covered with aluminium foil. The other side was irradiated with a bank of 6 UV-lights (model FL-40 BLB lamps 40 Watt tubes supplied by Toshiba Co., Japan, emission 320 - 400 nm) that had been equipped with window glass filters to eliminate radiation < 320 nm.
The distance of the skin from the light source was 10 cm. Irradiation continued for 70 minutes. The test sites were observed for reactions after 24 and 48 hours.

The intensity of reactions was graded from 0 - 8. The criteria and scoring system for assessment of dermal reactions were those of Draize. The scores for erythema and edema were totaled for the 5 test animals and this total was divided by 5 to give the primary irritation index.
Test substance: beta-Ionone (CAS-no. 14901-07-6), no data on purity
Conclusion: The test substance was considered to be non-irritant and

non-phototoxic to Guinea pig skin up to a concentration of 50%.

Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, well documented and acceptable for assessment

06-AUG-2004 (121)

5.2.2 Eye Irritation

Species: rabbit
Concentration: undiluted
Dose: .1 ml
Exposure Time: 72 hour(s)
Comment: not rinsed
No. of Animals: 3
Vehicle: none
Result: slightly irritating
EC classificat.: not irritating

Method: OECD Guide-line 405 "Acute Eye Irritation/Corrosion"
Year: 1992
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: Methods applied:
- European Economic Community (EEC), EEC Directive 84/449, Methods for the determination of toxicity, publication no. L 251, B.5: " Acute toxicity - eye irritation", September 19, 1984
- Organization for Economic Co-operation and Development (OECD), OECD Guidelines for Testing of Chemicals, Guideline No. 405: " Acute eye irritation / corrosion"; adopted February 24, 1987

Result: The treatment lead to the following effects at the different observation times (individual and mean scores).
For calculation of the means of opacity, iris, redness and chemosis only the readings of 24, 48 and 72 hrs were used.

Readings	1 hr	24 hrs	48 hrs	72 hrs	Mean
Cornea					
-Opacity	0,0,0	0,1,0	0,0,0	0,0,0	0.1
-Area	0,0,0	0,2,0	0,0,0	0,0,0	
Iris	0,0,0	0,1,1	0,0,0	0,0,0	0.2
Conjunctiva					
-Redness	2,2,2	1,2,2	0,2,2	0,0,0	1.0
-Chemosis	0,1,0	0,1,0	0,0,0	0,0,0	0.1
-Discharge	2,2,2	0,1,1	0,1,1	0,0,0	

Small retractions in the eyelid were observed at 24 hrs and later.

Test condition: TEST ANIMALS
Strain: White Vienna rabbits
Sex: 2 males, 1 female
Source: M. Gaukler, Offenbach, Germany
Weight at study initiation (mean): 3.27 kg (males) and 2.79 (females)

Controls: untreated eye
EXPOSURE
Single application to the conjunctival sac of the right eyelid (no wash out)
Post exposure period: 72 hrs

EXAMINATION
Readings: 1, 24, 48 and 72 hrs
Scoring system according to EEC and OECD criteria

Conclusion: After application into the conjunctival sac, the test substance led to slight corneal opacity and conjunctival chemosis in one animal after 24 hrs and to well-defined conjunctival redness after 1, 24 and 48 hrs. All effects were completely reversible within 72 hrs of observation.

Reliability: (1) valid without restriction
GLP guideline study

Flag: Critical study for SIDS endpoint

15-JUN-2004 (122)

Species: rabbit
Concentration: undiluted
Dose: .1 ml
Comment: not rinsed
No. of Animals: 3
Vehicle: none
Result: not irritating

Method: other: comparable to Draize method
Year: 1967
GLP: no
Test substance: other TS

Result: Eye irritation
(mean values of 3 animals)

Time	0	1	2	4	24	48	72 hrs
Cornea							
-Opacity	0	0	0	0	0	0	0
-Area	0	0	0	0	0	0	0
Pupil reaction	+	+	+	+	+	+	+
Conjunctiva							
-Redness	1	1	1	1	0	0	0
-Chemosis	0	0	0	0	0	0	0
-Discharge	-	0	0	0	0	0	0

Test condition: 0.1 ml of the compound was applied at full strength into the conjunctival sac of one eye of 3 rabbits. The untreated eye seved served as control. Observations of the eyes were made immediately and 1, 2, 4, 24, 48 and 72 hrs later. Fluorescein was injected to check corneal damage. Scoring of the eyes was done by the method of Draize (1955).
Scale used for scoring:
0 = normal, 1 = very slight, 2 = well defined,
3 = moderate, 4 = severe

Test substance: beta-Ionone, no further data on purity
Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, well documented and acceptable for assessment

15-JUN-2004 (120)

Species: rabbit
Concentration: 5 %
Dose: .1 ml
Comment: not rinsed
No. of Animals: 3
Vehicle: other: diethylphthalate
Result: not irritating

Method: other: comparable to Draize method
Year: 1967
GLP: no
Test substance: other TS

Result: Eye irritation
(mean values of 3 animals)

Time	0	1	2	4	24	48	72 hrs
Cornea							
-Opacity	0	0	0	0	0	0	0
-Area	0	0	0	0	0	0	0
Pupil reaction	+	+	+	+	+	+	+
Conjunctiva							
-Redness	1	0	0	0	0	0	0
-Chemosis	0	0	0	0	0	0	0
-Discharge	-	0	0	0	0	0	0

Test condition: 0.1 ml of the compound was applied at 5% concentration in diethylphthlate into the conjunctival sac of one eye of 3 rabbits. The untreated eye seved served as controls. Observations of the eyes were made immediately and 1, 2, 4, 24, 48 and 72 hrs later. Fluorescein was injected to check corneal damage. Scoring of the eyes was done by the method of Draize (1955).

Scale used for scoring:
0 = normal, 1 = very slight, 2 = well defined,
3 = moderate, 4 = severe

Test substance: beta-Ionone, no further data on purity
Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, well documented and acceptable for assessment

28-MAY-2004 (120)

5.3 Sensitization

Type: Guinea pig maximization test
Species: guinea pig
Concentration 1st: Induction 10 % intracutaneous
2nd: Induction 10 % occlusive epicutaneous
3rd: Challenge 40 % occlusive epicutaneous
No. of Animals: 9
Vehicle: other: acetone
Result: not sensitizing
Classification: not sensitizing

Method: OECD Guide-line 406 "Skin Sensitization"
Year: 1990
GLP: no data
Test substance: other TS

Result: No evidence of erythema or edema was noted in any test group after challenging test item at a concentration of 40% (w/v) in acetone. No reactions were noted at the challenge sites of control animals. Sensitization index was 0.0 for all test groups (0, 5, 10, 20 and 40%).

Test condition: TEST ANIMALS
9 female albino Heartley Guinea pigs were used (5 test and 4 control animals) in the main study purchased from Japan SLC, Inc.

ADMINISTRATION/EXPOSURE
Hair on the back of the animal was cut with an electric hair clipper and an electric shaver. 4 hours after depilation, based on the results of a sighting test, the concentrations of test material for the induction and challenge phases were selected. For the purpose of this study the test material was freshly prepared as follows:

Intradermal induction:
- 10% (w/v) in Freund's Complete Adjuvant
- 10% (w/v) in a mixture of Freund's Complete Adjuvant plus physiological saline (1:1)
- Freund's Complete Adjuvant plus physiological saline (1:1)

Topical induction:
- 10% (w/v) in Freund's Complete Adjuvant

Topical challenge:
- 40, 20, 10, 5 % (w/v) in acetone

The concentration, homogeneity and stability of the test formulations were not determined. The intensity of skin reaction was graded from 0 - 8. The criteria and scoring system for assessment of reaction were those of Draize. The scores for erythema and edema at 48 hour reading were totaled for the 5 test Guinea pigs (5 values) and this total was divided by 5 to give the sensitization index of the test material.

Test substance: beta-Ionone (CAS-no. 14901-07-6), no data on purity
Conclusion: According to the authors, beta-Ionone was classified as a non-sensitizer.
Reliability: (2) valid with restrictions
Guideline study, only English description of the original Japanese report available. Reduced number of animals used (5 and 4 per group instead of 10).
Flag: Critical study for SIDS endpoint
09-AUG-2004 (121)

Type: Open epicutaneous test
Species: guinea pig
Concentration 1st: 8 %
No. of Animals: 6
Vehicle: other: see TC
Result: not sensitizing

Method: other: Klecak et al., J. Soc. Cosmet. Chem., 28, 53-64, 1977
Year: 1985
GLP: no
Test substance: other TS

Remark: The results were taken from a review which discusses the Freund's Complete Adjuvant Test and the Open Epicutaneous Test. Data for about 300 fragrance raw materials were presented in tabular format.
Result: Ionone tested at a concentration of 8% was not a sensitizer.
Test condition: The applied protocol was descibed in a general manner:

TEST ANIMALS
Male and female guinea pigs, weighing 300-450 g, were used. Experimental groups of at least 6 guinea pigs for every concentration group were utilized. For controls, 12 animals are used.

ADMINISTRATION AND EXPOSURE
The test material was applied epicutaneously, uncovered and if possible and relevant dissolved, suspended or emulsified at concentrations of 30, 10, 3 and 1% or lower in ethanol, acetone, water, PEG or petroleum. Constant volumes of each concentration were applied with a pipette or syringe on standard areas of the clipped flank of each animal.

Pretest:
1 day before starting the induction procedure, the threshold irritating concentration of the test material was estimated. A single application of 0.025 ml of each test concentration (e.g. 100, 30, 10 and 3%) was simultaneously performed on the clipped skin (area: 2 sq.cm). Reactions were read 24 hours after the application.
Induction:
On day 1, application of 0.1 ml of the test material (at the respective concentration) was performed to an area measuring 8 cm2 on the clipped flank. The applications were repeated daily for 3 weeks or done 5 times weekly during 4 weeks. The application sites were left uncovered and the reactions were read 24 hours after each application or at the end of each week. The maximal non irritating and the minimal irritating concentrations were determined.

Challenge:
To determine whether or not a contact sensitization was induced all groups previously treated for 21 days as well as 10 untreated, or only pretreated with the vehicle, controls were tested on days 21 and 35 on the contralateral flank with the test material at the minimal irritating and some lower primary non irritating concentrations. The substance was applied at 0.025 ml with a pipette of each concentration to skin areas of 2 sq.cm.

EXAMINATIONS
The reactions were read after 24, 48 and/or 72 hrs.

EVALUATION CRITERIA
A test material was considered allergenic at a concentration to which at least 1 of 6 animals of the concentration group concerned showed positive reactions when non irritating

concentrations were used for challenge.
Test substance: Ionone (mixture of alpha- and beta-isomer); no further specification is provided.
Reliability: (4) not assignable
Secondary literature
23-AUG-2005 (123)

Type: other: human maximization test
Species: human
Vehicle: no data
Result: not sensitizing

Method: other: Kligman Maximization test
Year: 1967
GLP: no
Test substance: other TS

Method: Kligman AM (1966). J. Invest. Dermatol. 47, 369.
Result: None of the 25 volunteers showed a positive reaction (result: 0/25). Ionone was classified as a non-sensitizer.
Test condition: The Human Maximization test (Kligman, 1966) was used to study 20 fragrance compounds including Ionone.

The procedure was as follows:

Induction:
25 healthy adult volunteers were recruited.
To a designated area of skin a patch test was applied with 1.0 ml of 5% aqueous sodium lauryl sulfate (SLS) solution for 24 hrs to produce a moderate inflammatory reaction. Following removal and at the same site, a 48 hrs occlusive patch was applied with the test material in high test concentration in a compatible vehicle. No further information on test concentration given.
The last 2 steps were alternated for a total of 5 exposures of each which are accomplished over a period of of 15 days.
Following an additional 10-day rest period the induction phase was complete.

Challenge:
At the conclusion of the 10-day rest period, a new skin site was selected to which a 10% SLS solution was applied for one hour. It was then washed off and the test material was applied to this new pretreated area under an occlusive patch for 48 hrs before removal. The test area was examined immediately and again on each of 2 successive days. The reactions were then scored for each subject.

Test material:
Ionone was tested at a 8% dilution.
Test substance: Mixture of 97.5 % alpha-Ionone and 2.5 % beta-Ionone; no further data on purity.
Reliability: (4) not assignable
Documentation insufficient for assessment. Test substance consists mainly of alpha-Ionone.
28-MAY-2004 (124)

5.4 Repeated Dose Toxicity

Type: Sub-acute
Species: rat **Sex:** no data
Strain: no data
Route of administration: gavage
Frequency of treatment: daily
Post exposure period: no data
Doses: 500, 1000, 2000, 4000, 8000 mg/kg bw/day
Control Group: no data specified

Method: other
Year: 1975
GLP: no
Test substance: other TS

Result: MORTALITY

- Mortality expressed in %, body weight (bw) change determined between day 0 and day 5

Time	1	2	3	4	5	15 days	bw change
mg/kg bw							
8000	30	100					
4000	0	10	10	20	40	40	+8.7
2000						0	+14.4
1000						0	+19.0
500						0	+22.4

CLINICAL SYMPTOMS

1000 - 8000 mg/kg bw: sedation
500 mg/kg bw: slight sedation

LETHAL DOSES
(expressed in mg/kg bw)

	After 24 hrs	after 5 days	after 15 days
LD10	5550	2620	2620
LD50	> 8000	4110	4110
LD90	> 8000	6450	6450

Test condition: Rats received daily oral doses of the test substance for 5 days. Mortality, clinical symptoms and body weights were recorded. The LD10, LD50 and LD90 were calculated 5 and 10 days after the last application. No further information available.
Test substance: beta-Ionone, no further data on purity
Reliability: (2) valid with restrictions
Data from Handbook or collection of data; endpoints limited to mortality, body weight and clinical symptoms
09-NOV-2004 (125)

Type: Sub-acute
Species: rat **Sex:** male/female
Strain: Wistar
Route of administration: oral feed
Exposure period: 4 weeks
Frequency of treatment: continuously
Post exposure period: none
Doses: 1000, 5000, 15000 ppm
Control Group: yes, concurrent vehicle

NOAEL: = 1000 ppm
LOAEL: = 5000 ppm

Method: other: Range finding study
Year: 2004
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Remark: Study was a range finding study for a subsequent subchronic feeding study in rats (BASF project no. 50S0449/02057).
The study was conducted in accordance with the OECD Principles of Good Laboratory Practice and the GLP provisions of the German "Chemikaliengesetz" (Chemicals Act), with the exception of a QAU - check. Additionally the substance preparations were not checked analytically and no full report was provided.

Result: The following treatment related main findings were obtained:

- 15000 ppm (ca. 1090-1260 mg/kg bw/day):
Reduced food consumption (-44% at day 7 and -7% at day 28 in males; -38% at day 7 and -22% at day 28 in females), reduced water consumption (-29% at day 7 and -16% at day 29 in males; -7% at day 7 and -21% at day 28 in females), reduced body weight (-13% in males, -14% in females at day 28), reduced body weight gain (-28% in males and -40% in females at day 28), discolored urine in males and females, piloerection in single animals, increased absolute liver weight in males (+61%) and females (+48%), increased relative liver weights in males (+87%) and females (+74%), increased relative kidney weights in males (26%) and females (+18%)

- 5000 ppm (ca. 390-510 mg/kg bw/day):
Reduced food consumption in females (-10 to -14% from days 14-28), reduced body weight in females at day 28 (-12%), increased absolute liver weight in males (+44%) and females (16%), increased relative liver weights in males (+46%) and females (+33%), increased relative kidney weights in males (+15%)

- 1000 ppm (ca. 80-100 mg/kg bw/day): no relevant substance related abnormalities

No treatment related mortality occurred. Based on these results (significantly reduced food and water consumption at 15000 ppm, body weight data and organ weight determinations), test concentrations of 100, 1000 and 10000 ppm were selected as doses for the main study.

Test condition: Beta-Ionone was administered for 4 weeks to groups of Wistar rats (5 per sex) at dietary concentrations of 0, 1000, 5000 and 15000 ppm.
Food consumption, water consumption and body weight were determined once a week. The animals were examined for signs of toxicity or mortality at least once a day. At the end of the administration period the animals were subjected to gross-pathological assessment.

Test substance: beta-Ionone, purity 97.8%

Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, well documented and acceptable for assessment

15-NOV-2004 (126)

Type: Sub-chronic
Species: rat **Sex:** male/female
Strain: Wistar
Route of administration: oral feed
Exposure period: 3 months
Frequency of treatment: continuously
Post exposure period: none
Doses: 100, 1000, 10000 ppm
Control Group: yes, concurrent vehicle
NOAEL: = 1000 ppm
LOAEL: = 10000 ppm
NOEL : = 100 ppm

Method: OECD Guide-line 408 "Subchronic Oral Toxicity - Rodent: 90-day Study"
Year: 2004
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Result: ANALYSES

- Stability of the test substance: was demonstrated in the diet over a period of 49 days at room temperature
- Concentration control: correctness of the concentrations were confirmed. The recovery rates were within a range of 92.3% - 102.6% of the target concentrations.

TEST SUBSTANCE INTAKE

Dose	Conc. (ppm)	Intake (mg/kg bw/d)	
		Males	Females
Low	100	7.1	8.2
Mid	1000	71.8	83.0
High	10000	719.6	801.0

CLINICAL EXAMINATIONS

- Mortality: One female animal of the control group was sacrificed moribund on day 42.

- Clinical signs: single findings (one moribund female in control group, one male animal of the high dose group with alopecia) were assessed as being incidental and not substance-related.

- Food consumption: Food consumption of male and female animals in the high dose group was statistically significantly decreased on day 7 (-24.5%). As no further significant impairment was seen, this was assessed as being incidental.

- Water consumption: no changes

- Body weight data: In high dose males, body weight (day 7) and body weight change (day 7 and 14) was statistically significantly decreased. These findings were connected with the above-mentioned impaired food consumption and therefore assessed as being incidental.

- Food efficiency: In high dose males, food efficiency was statistically significantly decreased on day 7 and 28. Due to

the isolated occurrence and in connection with the above-mentioned impaired food consumption, this finding was assessed as being incidental.

- Ophthalmoscopy: No substance-related effects were obtained.

- Functional observational battery and motor activity measurement: All findings were assessed as being incidental, as they occurred in single animals, only, or were equally distributed between treated groups and controls.

- Estrous cycle determination: No substance-related effects were obtained.

CLINICAL PATHOLOGY

- Hematology: At the end of the administration period, prothrombin times were significantly shortened in the high dose females. No treatment-related effects were seen in the other hematological parameters of both sexes.

- Clinical chemistry: Enzyme examinations revealed marked increases in gamma-glutamyltransferase activities in the serum of the high dose animals of either sex after 3 months of test substance administration. No treatment-related changes were found in the other enzyme measurements.
Blood chemistry investigations showed increased calcium, total protein, albumin, globulins and cholesterol concentrations in the serum of the high dose male and female animals. However, the increase in albumin in the females of the high dose group was not statistically significantly different to the concurrent control, but was seen as a tendency towards higher values. Dose-dependent, statistically significantly increased inorganic phosphate levels were measured in the serum of all treated males. Although the increases in inorganic phosphate were statistically significantly different to the control value and showed a dose-response relationship, these isolated findings were assessed as being fortuitous since similar increases were not observed in the females. The differences were attributed to a low control group value and not to the test compound administered. No toxicologically relevant changes were observed in the other blood chemistry parameters.

- Thyroid hormone measurements: Slightly but significantly decreased thyroxine (T4) levels (-19%) were found in the high dose males. A relationship to treatment could not be excluded. The significant changes in the low and mid dose females were not regarded as treatment related as they occurred without a dose relationship.

Table: Thyroid hormone determinations

	T3 (nM)		T4 (nM)		TSH (µg/l	
Dose Groups	M	F	M	F	M	F
Control	1.31	1.49	55.2	30.7	7.1	5.8
Low	1.34	1.74	54.0	42.4**	7.6	5.0
Mid	1.36	1.44	60.6	37.3*	8.0	5.8
High	1.6	1.63	44.9**	33.1	7.2	5.9

* p < = 0.05, ** p < = 0.01

- Urinalyses: At the end of the study, an increased amount of ketones were excreted by the high dose males and females and in the urine specimens of the high dose females increased urobilinogen levels were measured. Microscopic examination of the urine sediments revealed increased numbers of degenerated transitional epithelial cells in the mid and high dose males as well as in the high dose females. Higher number of granular and epithelial cell casts was also observed in the urine specimens of the mid and high dose males. The test compound did not affect the other urine parameters.

- Sperm analysis: There were no treatment-related effects on sperm parameters.

PATHOLOGY

- Absolute organ weights: When compared to control group, the mean absolute weights of following organs were significantly increased:

Group	L	M	H
Liver	na (m,f)	na (m)	+65.3% (m)
		+10.2% (f)	+52.5% (f)
Kidneys	na (m,f)	na (m,f)	+22.7% (m)
			na (f)
Testes	na	na	+11.9%

L, M, H = low, mid, high dose
m, f = males, females
na = no abnormalities

All other mean absolute weight parameters did not show significant differences when compared to the control group.

- Relative organ weights (related to terminal body weight): When compared to control group 0, the mean relative weights of following organs were significantly decreased or increased:

Group	L	M	H
Liver	na (m,f)	+9.1% (m)	+73.4% (m)
		+7.6% (f)	+54.8% (f)
Kidneys	na (m,f)	na (m,f)	+28.0% (m)
			+10.6% (f)
Testes	na	na	+17.5%
Epididymides	na	na	+11.0%
Adrenal glands	na (m,f)	-11.1% (f)	na (m,f)

The decrease of the mean relative adrenal weight in females of the mid dose group was considered incidental.
All other mean relative weight parameters did not show significant differences when compared to the control groups.

- Gross lesions: All gross lesions occurred singly. In two high dose males, a unilateral pelvic dilation of the kidneys was observed.

- Histopathology:

Liver: A minimal to slight central hypertrophy of hepatocytes was noted in three mid dose males and in all high dose males and females. In addition, two high dose males showed a peripheral hypertrophy of hepatocytes. In the cytoplasm of these hypertrophic hepatocytes, myelinoid figures were noted. The myelinoid figures most likely represent arrays of concentric membranes (smooth endoplasmic reticulum).

Kidneys: Eosinophilic droplets were observed in some of the tubular epithelial cells in most of males. These eosinophilic droplets stained positive with Mallory-Heidenhain. The immunohistochemical examination of eosinophilic droplets with a specific antibody revealed alpha2u-globuline. The amount of this protein in tubular epithelial cells was higher in mid and high dose males than in controls. Chronic nephropathy was observed in a high incidence with higher degrees of severity in males of the high dose group. The macroscopically diagnosed unilateral pelvic dilation of the kidneys in two high dose males was confirmed histopathologically. The occurrence of this finding was considered incidental.

Thyroid gland: A flaky appearance of the colloid ("altered colloid") was noted in most untreated and treated males and females. The gradings of this finding, however, were increased in high dose males.

Stomach: In the region of the limiting ridge - the border between forestomach and glandular stomach - the epithelium showed prominent rete packs (diagnosed as "acanthosis") in two high dose males and in one mid and one high dose female. The occurrence of this finding was considered incidental.

Testes: The absolute and relative weights of testes were significantly increased in high dose males. Because there were no histopathological correlates for the increased weights, a substance-related effect seemed unlikely.

Epididymides: The relative weight of epididymides was significantly increased in high dose males. The absolute weight of epididymides did not show significant differences and there were no histopathological correlates for the increased weight. Therefore, this finding was considered due to the slightly (-4.2%) but not significantly decreased terminal body weight in these animals.

All other findings noted were either single observations or they were biologically equally distributed between control and treatment groups. All of them were considered to be incidental or spontaneous in origin and without any relation to treatment.

Test condition: TEST ORGANISM

Strain: CrlGlxBrlHan:WI (Supplier: Charles River, Germany)
Age at study initiation (day 0): 36 +/- 1 days
Weight at study initiation: about 150 g (males) and about 120 g (females)
Number of animals per group: 10 per dose and sex

ADMINISTRATION / EXPOSURE

- Duration of test/exposure: 90 days
- Test substance purity: 97.8% (HPLC)
- dosages were selected due to the results of a range finding study (BASF project no. 30S0449/02045)
- Vehicle: diet (Kliba maintenance diet mouse/rat "GLP")
- Preparation of test formulation: For each concentration, the test substance was weighed out and mixed with a small amount of food in a beaker. Subsequently, a premix was prepared in a mixer by adding an appropriate amount of food followed by mixing. Then corresponding amounts of food, depending on dose group, were added to this premix in order to obtain the desired concentrations. Mixing was carried out for about 10 minutes in a laboratory mixer.

- Stability of test substance in vehicle:
The stability of the test substance in the diet over a period of 49 days at room temperature was proven before the start of the study. Homogeneity and concentration control analyses of the test substance preparations were performed in samples of all concentrations at the start and at the end of the administration period.

CLINICAL OBSERVATIONS

- Clinical signs: The animals were examined for overt signs of toxicity or mortality twice a day from Mondays to Fridays and once a day on Saturdays, Sundays and public holidays. Additionally, further general clinical examinations were carried out daily. Detailed clinical observations outside the home cage in an open field (50 cm x 50 cm with sides of 25 cm high) were performed prior to the start of the administration period and weekly thereafter. The findings were ranked according to the degree of severity, if applicable.
The following parameters were examined: behavior during "handling", fur, skin, posture, salivation, respiration, activity/arousal level, tremors, convulsions, abnormal movements, impairment of gait, lacrimation, palpebral closure, exophthalmus, feces (appearance/consistency), urine and pupil size.

- Mortality: twice daily (Monday - Friday), once daily (Saturday and Sunday)
- Body weight: before the start of administration, thereafter once weekly
- Food consumption: once weekly
- Water consumption: daily
- Food efficiency: was calculated based upon individual values for body weight and food consumption.
- Intake of test substance: was calculated based upon individual values for body weight and food consumption.

- Ophthalmoscopic examination: Prior to the start of the administration period the eyes of all animals were examined for any changes using an ophthalmoscope after administration of a mydriatic. At the end of the study, the animals of the control and high dose group were examined.

- Functional observational battery (FOB): was performed towards the end of the study, starting at about 10.00 a.m.. The FOB started with passive observations without disturbing

the animals, followed by removal from the home cage, open field observations in a standard arena and sensorimotor tests as well as reflex tests. The findings were ranked according to the degree of severity, if applicable.
-- Home cage observations: The animals were observed in their closed home cages; any disturbing activities were avoided during these examinations in order not to influence the behavior of the animals. Attention was paid to posture, tremor, convulsions, abnormal movements, impairment of gait and general observations.
-- Open field observations: The animals were transferred to a standard arena (50 cm x 50 cm with sides of 25 cm high) and observed for at least 2 minutes. The following parameters were examined: behavior when removed from cage, fur, skin, salivation, nose discharge, lacrimation, eyes / pupil size, posture, palpebral closure, respiration, tremors, convulsions, abnormal movements / stereotypics, impairment of gait, activity/arousal level, feces, urine and number of rearings.
-- Sensorimotor tests/reflexes: The animals were removed from the open field and subjected to following sensorimotor or reflex tests: approach response, touch response, vision, pupillary reflex, pinna reflex, audition, coordination of movements, behavior during, vocalization, pain perception, grip strength of forelimbs, grip strength of hindlimbs, landing foot-splay test

- Motor activity assessment: was measured on the same day as FOB was performed. The measurement was performed in the dark using the Multi-Varimex-System with 4 infrared beams per cage. During the measurement the animals were kept in Polycarbonate cages with absorbent material. The measurements started at about 2.00 p.m. and the number of beam interrupts were counted over 12 intervals, each lasting 5 minutes. The period of assessment for each animal started when the first beam was interrupted by pushing the cage into the rack. Measurements ended exactly 60 minutes thereafter.

- Estrus cycle determination: Vaginal smears for cycle determination were prepared in the morning and evaluated from day 63 until day 91 of the study. The differentiation was conducted to following stages:

Cycle stage	Appearance in vaginal smear
-Diestrous	Leucocytes, few nucleated, epithelial cells
-Proestrous	Single leucocytes, many nucleated and few horny epithelial cells
-Estrous	Only horny epithelial cells
-Metestrous	Leucocytes, some horny epithelial cells and some nucleated epithelial cells

CLINICAL PATHOLOGY

Blood was taken from the retroorbital venous plexus in the morning from fasted animals without anesthesia. At necropsy specimen were sampled from fasted anesthetized male animals in a randomized sequence for sperm analyses. The following examinations were carried out in 10 animals per test group and sex at the end of the application period.

- Hematology: The following parameters were determined in blood with EDTA-K3 as anticoagulant using a particle counter: leukocytes, erythrocytes, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelets, differential blood count, reticulocytes. Prothrombin time was determined using a ball coagulometer.

- Clinical chemistry: An automatic analyzer was used to examine the following clinicochemical parameters.
alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, serum gamma-glutamyltransferase, sodium, potassium, chloride, inorganic phosphate, calcium, urea, creatinine, glucose, total bilirubin, total protein, albumin, globulins, triglycerides, cholesterol, magnesium.

- Thyroid hormone measurements: Hormones were determined with radioimmunoassys using commercially available test kits and a gamma-counter. Total triiodothyronine (T3), total thyroxine (T4) and thyroid stimulating hormone (TSH) were measured.

- Urinalysis: With the exception of volume, color, turbidity, sediment examination and the specific gravity, all the uine constituents were determined semiquantitatively using test strips and a reflection photometer. The specific gravity was determined using a urine refractometer. The sediment was evaluated microscopically. The following examinations were carried out: volume, color, turbidity, pH, protein, glucose, ketones, urobilinogen, bilirubin, blood, specific gravity, sediment.

- Sperm parameters: Immediately after necropsy and organ weight determination the right testis and cauda epididymis were taken from all male animals. The following parameters were determined: sperm motility, sperm morphology, sperm head count (cauda epididymis), sperm head count (testis).

PATHOLOGY

- Necropsy: The animals were sacrificed by decapitation under CO2 anesthesia. The exsanguinated animals were necropsied and assessed by gross pathology.

- Organ weights: The following weight parameters from all animals sacrificed at scheduled dates were determined:
Anesthetized animals, liver, kidneys, adrenal glands, testes, epididymides, ovaries, uterus, spleen, brain, heart, thymus, prostate gland

- Histopathology: Left testis, left epididymis and both ovaries were fixed in Bouin's solution. After fixation, the organs were embedded in paraplast. The following organs were fixed in 4% formaldehyde solution, histopathologically processed and examined by light microscopy:
All gross lesions, salivary glands (glandula mandibularis and glandula sublingualis), esophagus, stomach (forestomach and glandular stomach), duodenum, jejunum, ileum, cecum, colon, rectum, liver, pancreas, brain, pituitary gland, sciatic nerve, spinal cord (cervical, thoracic and lumbar cord), eyes,

adrenal glands, thyroid glands, parathyroid glands, trachea, lungs, pharynx, larynx, nose (nasal cavities), aorta, heart, bone marrow (femur), lymph nodes (mandibular and mesenteric), spleen, thymus, kidneys, urinary bladder, oviducts/uterus/vagina, prostate gland, seminal vesicles, female mammary gland, skin, skeletal muscle, sternum with marrow, femur with knee joint, extraorbital lacrimal glands

STATISTICAL METHODS

Means and standard deviations of each test group were calculated for several parameters. Further statistical analyses were performed.

- Dunnett test:
Food and water consumption, body weight change, food efficiency

- Kruskall-Wallis test:
Feces, rearing, grip strength length forelimbs, grip strength length hindlimbs, landing foot-splay test, motor activity, clinical pathology, pathological weight parameters (if p-value < = 0.05 Wilcoxon test was additionally performed)

- Fishers exact test:
Urinalysis, except volume, color, turbidity and specific gravity; abnormal sperm > 4%

- Wilcoxon test
Total spermatids/g testis, total sperm/g cauda epi., % motility

Test substance: beta-Ionone, purity 97.8%

Conclusion: The administration of beta-Ionone over a period of 3 months at dietary concentrations of 100, 1,000 and 10,000 ppm lead to signs of general systemic toxicity. Target organs were liver, kidneys and thyroid glands. Regarding estrous cycle determination and sperm evaluation, no influence on the reproduction system was obtained. Moreover, no signs of neurotoxicity were observed during functional observational battery as well as measurement of motor activity performed towards the end of the administration period.

Thus, the no-observed-effect-level (NOEL) under the conditions of the present study was 100 ppm for both sexes (about 7.1 and 8.2 mg/kg bw/day for males and females) based on adaptive liver effects in both sexes and minor urine findings in males at 1000 ppm which correspond to a dosage of 72 and 83 mg/kg bw/day for males and females
(no-observed-adverse-effect-level, NOAEL).
The lowest-observed-adverse-effect-level (LOAEL) was found at 10 000 ppm (720 and 801 mg/kg bw/day for males and females) due to liver, kidney and thyroid findings in both sexes.

Reliability: (1) valid without restriction
GLP guideline study

Flag: Critical study for SIDS endpoint

22-AUG-2005 (127)

Type: Sub-chronic
Species: rat **Sex:** male/female
Strain: other: FDRL

Route of administration: oral feed
Exposure period: 90 days
Frequency of treatment: continuously
Post exposure period: no data
Doses: 11.6 and 13.1 mg/kg bw/day for males and females, respectively
Control Group: yes, concurrent no treatment
NOAEL: 11.6 mg/kg bw

Method: other
Year: 1965
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Remark: Dose selected so as to be at least 100 times the maximum estimated human dietary level.
Result: The treatment did not lead to any adverse effects.
Test condition: A 90 day study with 15 FDRL rats (per sex per dose), weighing 82.1 +/- 2.3 g for males and 77.1 +/- 2.3 g for females were used. Each test substance was diluted in cotton-seed oil in a concentration sufficient to provide the predetermined dosage in 2% of the diet. Dosages were administered on a uniform body weight basis by biweekly adjustments of the concentration of the test material in the cotton-seed oil (expected dose of beta-Ionone in the diet 11.4 mg/kg bw/day). Observations included body weight and food consumption. In addition, haematological and blood chemical determinations were made on 8 rats of each sex at a 6 week period and in all rats at 12 weeks. The tests were terminated at 90 days. All animals were sacrificed and a gross necropsy was carried out. At necropsy, liver and kidney weights were recorded and the following organs from half the animals in each group were taken for histological examination: liver, kidneys, stomach, small andlarge intestines, spleen, pancrease, heart, lungs, bone marrow, muscle, brain, spin cord, bladder, adrenals, thyroid, pituitary, gonads, salivary glands, and lymph nodes.
Test substance: beta-Ionone; typical of the commercial grade; no further data
Reliability: (3) invalid
Significant methodological deficiencies. Restrictions: only one dose, documentation of results insufficient
15-NOV-2004 (128)

Type: Sub-chronic
Species: rat **Sex:** no data
Strain: no data
Route of administration: oral feed
Exposure period: 12 weeks
Frequency of treatment: continuously
Post exposure period: no data
Doses: no data
Control Group: no data specified
NOAEL: 11.4 mg/kg bw

Method: other
Year: 1967
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Result: It was reported that 11.4 mg/kg bw did not lead to any effects. No further data.

Test condition: No further information on test conditions.
Test substance: beta-Ionone, no further data on purity
Reliability: (3) invalid
Significant methodological deficiencies. Documentation of methods and results insufficient
15-NOV-2004 (129)

Type: Sub-chronic
Species: rat **Sex:** male/female
Strain: no data
Route of administration: oral feed
Exposure period: 17 weeks
Frequency of treatment: continuously
Post exposure period: no data
Doses: 0.1; 0.25; 1 % in the diet (ca. 50; 125; 500 mg/kg bw/day)
Control Group: yes

Method: other
Year: 1967
GLP: no data
Test substance: other TS

Result: No adverse effects were observed on growth, appearance, food intake, haematology, final body weight, organ weights or macroscopic appearance of organs of rats at all dose levels. However, microscopic examination revealed swelling of the hepatic parenchymal cells. This swelling was dose-dependent, being slight to moderate in the highest level, slight in the intermediate level and very slight at the low level.
Test condition: Groups of 10 male and 10 female Osborne-Mendel rats were maintained for 17 weeks on diets containing "Ionone Standard" (60% alpha-Ionone and 40% beta-Ionone) at levels of 0, 1000, 25000 and 10000 ppm (approx. equivalent to 50, 125 and 500 mg/kg bw/day, when a feed consumption of 20 g per day and a body weight of 400 g is assumed).
Test substance: Mixture of 60 % alpha-Ionone and 40 % beta-Ionone; no further data on purity.
Reliability: (3) invalid
Documentation insufficient for assessment
15-NOV-2004 (130) (131)

Type: Sub-chronic
Species: rat **Sex:** male/female
Strain: Sprague-Dawley
Route of administration: oral feed
Exposure period: 90 days
Frequency of treatment: continuously
Post exposure period: no data
Doses: 10 and 100 mg/kg bw/day
Control Group: yes, concurrent no treatment
NOAEL: 10 mg/kg bw

Method: other
Year: 1983
GLP: no data
Test substance: other TS

Result: 10 mg/kg bw/day
No abnormalities

100 mg/kg bw/day
Reduced body weight gain, food consumption and serum glucose concentration, increased water intake, mild renal changes, no histological changes were evident in the kidneys and liver.

Test condition: Groups of 15 Sprague-Dawley rats per sex were fed a diet containing the test substance for 90 days. A control group was included. Renal functions and hematological studies were performed mid way through the treatment period and at the end of the study. In addition, the following data was collected: body weights, food intakes, water intakes, and organ weights.

Test substance: beta-Ionone, indication of purity is missing

Reliability: (4) not assignable
Secondary literature

15-NOV-2004 (132)

5.5 Genetic Toxicity 'in Vitro'

Type: Ames test
System of testing: Salmonella typhimurium TA98, TA100, TA1535, TA1537
Concentration: 1, 3, 10, 33, 50, 100, 180 µg/plate
Cytotoxic Concentration: 50 µg without S-9, 180 µg with S-9
Metabolic activation: with and without
Result: negative

Method: other: according to Haworth et al. (1983) and Zeiger and Drake (1980)
Year: 1986
GLP: no data
Test substance: other TS

Method: Approach and rationale for the testing were described in detail in:
- Haworth S, Lawlor T, Mortelmans K, Speck W and Zeiger E (1983). Salmonella mutagenicity test results for 250 chemicals. Environ. Mutagen. 5 (Suppl 1), 3-142

- Zeiger E and Drake JW (1980). An environmental mutagenesis test development programme. In: Montesano R, Bartsch H, Tomatis L (eds.). Molecular and cellular aspects of carcinogen screening tests. Lyon, IARC Scientific Publications No. 27, 303-313

Remark: The laboratory which performed the test was EG & G Mason Research Institute (USA).

Result: The results for the 270 test chemicals in the publication were presented in tabular format.

SOLUBILITY
No test substance precipitation was reported.

TOXICITY
A strong bacteriotoxic effect (complete clearing of background lawn) was seen in TA100 at 50 µg/plate without S-9 mix. A weak bacteriotoxic effect (slight clearing of background lawn) was observed at 50 µg/plate in TA1535, TA1537 and TA98 with S-9 mix and in all strains at 180 µg/plate with S-9 mix.

MUTAGENICITY
An increase in the number of his+ revertants was not observed

either without S-9 mix or after the addition of a metabolizing system (see table below). The positive controls showed the expected results.

REVERSION FREQUENCIES
Only the final test data were presented in the publication (however, it was indicated that if the data were considered equivocal or weakly positive, the complete data set was shown).

Results as mean values from 3 plates

Pos = positive control (see TC)
S-9(R) = rat liver S-9
S-9(H) = hamster liver S-9
s = slight clearing of background lawn
t = complete clearing of background lawn

Strain	TA100			TA1535		
Dose (µg)	-S-9	+S-9(H)	+S-9(R)	-S-9	+S-9(H)	+S-9(R)
0	140	144	136	22	9	7
1	137			23		
3.3	115	130	135	26	11	11
10	136	124	103	25	10	8
33	132	112	127	15	15	8
50	t			18s		
100		136	117		10	9
180		130s	102s		15s	6s
Pos	1399	706	901	1316	64	108

Strain	TA1537			TA98		
Dose (µg)	-S-9	+S-9(H)	+S-9(R)	-S-9	+S-9(H)	+S-9(R)
0	9	8	14	23	29	33
1	10			23		
3.3	11	8	11	24	27	31
10	9	8	10	23	27	27
33	10	7	9	24	30	31
50	7s			18s		
100		9	10		33	30
180		7s	5s		31s	25s
Pos	527	110	101	1442	1171	876

Test condition: SYSTEM OF TESTING

- Bacterial strains:

Salmonella strains TA1535, TA1537, TA98 and TA100 were obtained from Dr. B. Ames (University of California, Berkely, CA, USA). All bacterial cultures were grown overnight for 2-15 hrs at 37°C on a shaker and their phenotypes were analyzed as recommended by Ames et al. (Ames et al., 1975: Methods for detecting carcinogens and mutagens with the Salmonella/mammalian microsome mutagenicity test. Mut. Res.

31, 347-364).

- Metabolic activation system:

Male Sprague-Dawley rats and male Syrian hamsters were routinely used for the preparation of the liver fractions. Aroclor 1254 (200 mg/ml in corn oil) was administered i.p. at 500 mg/kg bw 5 days prior to decapitation. The livers were removed, washed in ice-cold 0.15 M KCl, and minced and homogenized in a Potter Elvjehem apparatus (3 ml of 0.15 M KCl per g of wet tissue). The S-9 fraction was dispensed into freezing ampuled and stirred in a -70°C freezer or in liquid nitrogen. The S-9 mix was prepared immediately prior to each assay (composition per ml: S-9 fraction 0.1 ml; 0.04 M MgCl2 0.02 ml; 1.65 M KCl 0.02 ml; 0.04 M NADP 0.1 ml; distilled water 0.56 ml)

- Preincubation Test

To each of 13 x 100 mm test tubes maintained at 37°C were added in the following order: 0.5 ml S-9 mix or 0.1 M PO4 buffer (pH 7.4), 0.05 ml of the overnight culture, and 0.05 ml solvent or chemical dilution. The mixture was mixed and incubated without shaking at 20°C for 20 min, at which time 2.5 ml of molten (45°C) top agar supplemented with 0.5 mM L-histidine and 0.5 mM D-biotin were added. The contents of the tubes were mixed and poured onto 25 ml of minimal glucose bottom agar in 15 x 100 mm plastic petri dishes. When the top agar had soldified, the plates were inverted and incubated at 37°C for 48 hrs.

ADMINISTRATION

Number of replicates: at least 5 dose levels with 3 plates per dose level

Preliminary dose setting: the test chemicals were in general initially tested with strain TA100 +/- S-9 mix over a wide dose range up to 10 mg/plate or less when solubility problems occurred. Bacteriotoxicity was evidenced. Non-toxic chemicals were tested in an initial experiment up to 10 mg/plate or to a level determined by the solubility of the test substance. Toxic chemicals were tested up to a high dose which exhibited some degree of toxicity.

Negative controls: vehicle controls (DMSO)
Positive control groups and treatment:
Without S-9 mix:
- sodium azide (TA1535 and TA100): 2.5 µg/plate
- 4-nitro-o-phenylendiamine (TA98): 12 µg/plate
- 9-aminoacridine (TA1537): 80 µg/plate
With S-9 mix:
2-aminoanthracene (TA1535, TA100, TA1537, TA98): 1.5 µg/plate (rat) and 0.75 µg/plate (hamster)

GLP: In the publication, it is not mentioned whether the study was conducted formally according to Good Laboratory Practice requirements.

CRITERIA FOR EVALUATION

The test chemical was considered positive if the following criteria were met:
- A dose-related and reproducible increase in the number of revertant colonies, even if the increase was less than twofold
- A test substance was considered nonmutagenic when no increase in the number of revertants were elicited
- Questionable response: when there was an absence of a clear-cut dose-related increase in revertants or when the response was of insufficient magnitude to support a determination of mutagenicity

Test substance: beta-Ionone (CAS-no. 14901-07-6), purity: 98%
Conclusion: The test substance beta-Ionone was not mutagenic in the Ames test under the conditions chosen.
Reliability: (2) valid with restrictions
Comparable to guideline study with acceptable restrictions. Restriction: only 4 bacterial strains used instead of 5 as recommended by OECD TG 471.
Flag: Critical study for SIDS endpoint
15-JUN-2004 (133)

Type: Ames test
System of testing: Salmonella typhimurium TA98, TA100, TA1535, TA1537;
Concentration: 3 µmol/plate (= 576 µg/plate)
Cytotoxic Concentration: no data
Metabolic activation: with and without
Result: negative

Method: other: according to Ames et al. (1975). Mut. Res. 31, 347
Year: 1980
GLP: no data
Test substance: other TS

Result: Result in tabular format.
Beta-Ionone was not mutagenic to Salmonella typhimurium strains TA98, TA100, TA1535 or TA1537 in an Ames test with and without metabolic activation at a concentration of 3 µmol/plate (= 396 µg/plate).
No further details given.
Test condition: SYSTEM OF TESTING

Salmonella strains TA98, TA100, TA 1535 and TA1537 were obtained directly from Dr B Ames (University of California, Berkeley, CA, USA).
Revertants were scored on glucose minimal salts medium supplemented with 0.05 µmol histidine and 0.05 µmol biotin. Plates used for viable counts contained 10 µmol histidine and 0.05 µmol biotin. The experiments were carried out as described by Ames et al. (see above).
The following controls were made for each experiment:
- the viable count was determined;
- the number of spontaneous revertants was measured;
- the presence of the rfa-mutation was checked by crystal violet inhibition;
- the presence of the plasmid pKM101 in strains TA98 and TA100 was checked by resistance to ampicillin;
- the response to the positive controls N-methyl-N'-nitro-N-nitrosoguanidine (not requiring metabolic activation) and 2-aminoanthracene (requiring metabolic activation) was checked.

Metabolic activation system:
S-9 fractions for metabolic activation were prepared as described by Ames et al. (see above). Aroclor 1254 or 3-methylcholanthrene, both suspended in corn oil, were used as inducers in male Sprague-Dawley rats (Aroclor 1254: 500 mg/kg bw i.p. for 5 days; 3-methylcholanthrene: 20 mg/kg bw for 3 days). Full details as to the preparation of the S-9 mix are in the publication.

ADMINISTRATION
The substances were tested in spot tests using the four strains. Each substance was tested at 3 µmol/plate unless or otherwise indicated in the publication. Most compounds, including beta-Ionone, were dissolved in ethanol for incorporation into the plates. In absence of a background lawn of bacteria on the plates (indicationg toxicity) the test was repeated with a lower concentration. Substances giving an uncertain result in the spot tests were tested quantitatively at four concentration levels (up to 30 µmol/plate) [according to the table beta-Ionone was tested only at 3 µmol/plate].

Test substance: beta-Ionone.
All test substances including beta-Ionone were checked for purity using TLC, GC and NMR. Compounds containing more than 3% impurities were purified using preparative LC, recrystallisation and distillation. The structures of the test compounds were confirmed by NMR.

Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, well documented and acceptable for assessment. Restrictions: only 4 Salmonella strains used instead of 5 (as required by OECD TG 471), one dose level (5 required by OECD TG 471).
As beta-Ionone was not mutagenic with or without metabolic activation, this negative result is only stated summarily, which, however, is regarded as a valid procedure.

Flag: Critical study for SIDS endpoint

23-AUG-2005 (134)

Type: other: umu-test
System of testing: Salmonella typhimurium TA1535/pSK1002
Concentration: 200 µl/tube (ca. 190 µg/tube)
Metabolic activation: with and without
Result: positive

Method: other: method originally developed by Oda Y and Nakamura S et al. (1985). Mut. Res. 147, 219-229
Year: 1991
GLP: no
Test substance: other TS

Method: Induction of SOS repair. The procedure uses Salmonella typhimurium TA1535 which involves a plasmid (pSK1002) carrying a fused gene umuC'-'lacZ. In this gene, the umu operon is induced by DNA-damaging agents. The intensity of DNA-repair gene is measured by beta-galactosidase activity which is produced from the fused gene.

Result: Results only described in tabular format.
positive effects
2 hours reaction time:
umu test results [(A-B)/B values without S9 and with S9,

resp.] at 492.6 ug/ml/OD600 were 5.73 and "kill" (cells were killed), resp. No further details given.

Long term reaction:
350 µg/ml/OD600 (100 mg/l):
umu test reactions at 2,4,6 and 24 hrs with S9 were -,-,-,++, resp. No further details given.

Test condition: Salmonella typhimurium TA 1535/pSK1002 was used for this work. Bacteria were grown in Luria broth (LB broth) and in TGA media which were supplemented with 20 mg/l ampicillin. S9 fraction was prepared from livers of male rats pretreated with phenobarbital and 5,6-benzoflavone. Overnight cultivation of the test strain in the LB broth was diluted 50-fold into TGA medium and was incubatedat 37°C for 2 hours with 145 rpm reciprocal shaker. The culture (TGA medium) was subdivided into 4.8 ml portions in test tubes, and 0.2 ml of the test compound was added to each tube. Then, either 1.0 ml of 0.1 M phosphate buffer (pH 7.4) or S9 mixture containing 100 µl of S9 microsomal fraction for metabolic activation was added. After 2 hours of incubation at 37°C with shaking, beta-galactosidase activity in the cells was assayed.
The bacterial density was measured at OD600. 0.4 ml fractions of the culture were diluted with 3.6 ml of buffer, and the bacterial cells were made permeable to the chromogenic substrate for beta-galactosidase by adding 100 µl sodium dodecyl sulfate (SDS) and 20 µl chloroform, and then mixing vigorously. The enzyme reaction was initiated by the addition of 0.8 ml of 2-nitrophenyl-B-D-galactopyranoside solution (4 mg/ml in 0.1 M phosphate buffer, pH 7.0) at 28°C. After 15 minutes, the reaction was stopped by adding 2 ml of 1 M Na2CO3, and the absorbance at OD420 and OD550 was measured by spectrophotometer. The induction of beta-galactosidase against the solvent did not vary with the time course. The indicated value of blank in each time was set to be the base value (B). Net genotoxicity (beta-galactosidase) of the sample was calculated by subtracting this value (B) from the genotoxicity (A) of the sample in a response time. The judgment of genotoxicity in the umu-test was as follows: The chemical whose value of the (A-B)/B gives over two was decided to have strongly positive genotoxicity. Hence, the following rank of genotoxic intensity was set. (A-B)/B > 2.0. = ++ (strongly positive); 2.0 > (A-B)/B > 1.0 = + (positive); 1.0 > (A-B)/B > 0.5 +-(weakly positive); 0.5 > (A-B)/B - (negative).

Test substance: Ionone, no further information given (CAS no. 8013-90-9, mixed isomers).

Reliability: (3) invalid
Significant methodological deficiencies. No validated method. No substance specification given, test concentrations not clear, documentation of results insufficient

24-AUG-2005 (135)

Type: other: spore rec- assay
System of testing: B. subtilis strains M45 (rec-) and H17 (rec+)
Concentration: 20 µg/disk
Metabolic activation: without
Result: positive

Method: other: Hirano et al. (1982). Mut. Res. 97, 339-347
Year: 1986
GLP: no

Test substance: other TS

Method: The method detects DNA damage by differences in growth inhibition zones (M45-H17).

Result: Results given in tabular format.
Inhibition zone M45 and H17 were 22 mm and 18 mm, resp. (difference = 4 mm). Result was judged to be positiveat 20µl per disk.

Test condition: The spore plate rec-assay with strains M45 (rec-) and H17 (rec+) was conducted. and germcidal activity was observed in test materials.

A small quantity of proliferated bacteria of the given strains was shake-cultured overnight in B-2 liquid broth and 1 ml of 50 % glycerin solution was added to 3 ml of the liquid broth to be cryopreserved at minus 80°C. After 0.1 ml bacteria suspension of H17 or M45 strains overnight cultured in the B-2 liquid broth was spread on the surface of tailored Schaeffer culture medium, the H17 strain was cultured for 3 days at 37°C and the M45 strain for 5 days at 37°C. Spores obtained were collected in mineral salt solution, washed with MM through centrifugation, re-suspended with MM and supplied with lysozyme to be kept at 37°C for 30 minutes. Next, sodium dodecyl sulfate was added to the solution to become finally a 1% solution to be kept at 37°C for 30 minutes. Each group of spores obtained was washed 5 times with distilled water through centrifugation and suspended in distilled water to be finally kept at 4°C.

Liquid agents were diluted with DMSO. The administration of the test substance was done with a quantity immersible into a round filter paper that is, 20 µl/disk. Each stock solution of 0.1 ml of the test substances was inoculated onto the B-2 agar-medium and kept at 37°C for 24 hours to be finally checked about its sterility. DMSO and mitomycin C (MMC) were used as controls.

B-2 agar-broth was heated and dissolved to be kept at 45°C and 10 ml obtained was supplied with o.1 ml suspension including spores of H17 or M45 strains (approx. 2 x 10E5 cells/ml). Each of them was injected into a plate and kept overnight at 4°C after the agar was solidified. On the next day, a round filter paper was placed in the middle of the plate and 20 µl of the test substance in double-serial diluted solution was immersed in the paper to be cultured at 37°C. The diameters of growth inhibition zones of the both strains generated after 24 hours were measured and compared.

Substances whose diameters of growth inhibition circles of the M45 strain are by 4mm and over bigger than those of the H17 strain were considered positive on the three and more spots of their dose response curves obtained from 4 to 5 spread areas.

Test substance: Ionone (purchased from Tokyo Kasei Industrial), no further information on specifity given (CAS-no. 8013-90-9, mixed isomers)

Reliability: (3) invalid
Significant methodological deficiencies. No validated method. No substance specification given, documentation of results insufficient

24-AUG-2005 (136)

Type: Bacterial reverse mutation assay
System of testing: E. coli WP2 uvrA
Concentration: 2.5-20 mg/plate
Cytotoxic Concentration: > 20 mg/plate
Metabolic activation: without
Result: negative

Method: other: Ishii Y and Kondo S (1975). Mut. Res. 27, 27-44; Brusick DJ et al. (1980). Mut. Res. 76, 169-190
Year: 1986
GLP: no
Test substance: other TS

Result: Results given intabular format. Ionone was judged to be negative (ratio 0.8). No toxicity on cells at maximal doses.
Test condition: The test was conducted with E. coli WP2 uvrA (trp-). A glucose minimal medium which was obtained by adding glucose by 2% and Bacto-agar by 1.5% to a Vogel-Bonner E medium was used.

Layering 0.6% soft agar added with 1/10 of 0.5mM L-tryptophan solution was kept at 45°C. Then, 0.1ml E. coli WP2 uvrA suspension (1-2 x 10E9 cells/ml) which was under a logarithmic growth phase in the preculture and 0.5ml phosphate buffer (pH 7.4) were added to obtain 2ml soft agar and mixed with 0.1ml solution of test substance in a prescribed concentration. The sample obtained was layered onto the prepared plate of glucose minimal medium and uniformly spread to be solidified. The number of colonies appeared after 2-day incubation at 37°C was counted, and the number of revertants per plate was determined by averaging the number of colonies on 3 plates.
Test substance: Ionone (purchased from Tokyo Kasei Industrial), no further information on specifity given (CAS-no. 8013-90-9, mixed isomers).
Reliability: (3) invalid
Significant methodological deficiencies. No substance specification given, only one bacterial strain used, no S9-mix, documentation of results insufficient.
22-AUG-2005 (136)

5.6 Genetic Toxicity 'in Vivo'

Type: Micronucleus assay
Species: mouse **Sex:** male
Strain: NMRI
Route of admin.: i.p.
Exposure period: single administration
Doses: 250; 500; 750 mg/kg bw
Result: negative

Method: OECD Guide-line 474 "Genetic Toxicology: Micronucleus Test"
Year: 2003
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: Beta-Ionone was tested for chromosomal damage (clastogenicity) and for the ability to induce spindle poison effects (aneugenic activity) in NMRI mice using the micronucleus test method. For this purpose, the test substance, dissolved in

olive oil, was administered once intraperitoneally to male animals at 3 dose levels.

Result: ANALYTICAL RESULTS
The stability of the test substance at 4°C in the vehicle (homogenous solution) over a period of 4 days was verified analytically.

MORTALITY
No mortality occurred in all groups.

CLINICAL SIGNS
The single administration of the test substance at 750 and 500 mg/kg bw led to evident signs of toxicity in all treated animals (poor general state, irregular respiration, squatting posture) which were reversible after 2 days. At the lowest doses only minor signs of clincal toxicity were observed after 2 and 4 hours of administration of the test substance (squatting posture).

EFFECT ON PCE/NCE RATIO
No inhibition of erythropoiesis, determined from the PCE/NCE ratio was detected. The vehicle and the the positive control substances, CPP and VCR, caused no evident signs of toxicity.

Mean number of PCEs and NCEs:

Interval		24 hrs	48 hrs
	PCEs	NCEs	NCEs
Vehicle	10,000	4,594	3,439
250 mg/kg bw	10,000	3,755	
500 mg/kg bw	10,000	4,646	
750 mg/kg bw	10,000	2,476	2,804
CPP (20 mg/kg bw)	10,000	3,920	
VCR (0.15 mg/kg bw)	10,000	5,374	

Mean number of PCEs containing MN per 1,000 PCE at 24 hrs:
(differentiation between small and large micronuclei)

	Small	Large	Total
Vehicle	1.3	0.1	1.4
250 mg/kg bw	1.6	0.0	1.6
500 mg/kg bw	1.7	0.1	1.8
750 mg/kg bw	1.2	0.0	1.2
CPP (20 mg/kg bw)	10.9	0.2	11.1 (p <= 0.01)
VCR (0.15 mg/kg bw)	35.7	11.5	47.2 (p <= 0.01)

Mean number of PCEs containing MN per 1,000 PCE at 48 hrs:
(differentiation between small and large micronuclei)

	Small	Large	Total
Vehicle	0.7	0.0	0.7
750 mg/kg bw	1.0	0.0	1.0

STATISTICAL EVALUATION
The administration of the test substance did not lead to any statistical significant increase in the number of polychromatic erythrocytes containing either small or large micronuclei. The rate of micronuclei was nearly the range of the concurrent negative control in all dose groups and within the range of the historical control data (mean 1.6,

min. 0.3, max. 3.3, SD 0.6; n=393). The positive controls led to the expected increases in micronuclei (either small or large).

Test condition: TEST ORGANISM
Male healthy Crl:NMRI mice (breeder: Charles River Deutschland GmbH, GER) with a mean weight of about 29 g (with an age range of about 5-8 weeks according to the information of the breeder), 5 animals per dose and group

ADMINISTRATION
Vehicle: olive oil (quality: Ph.Eur/DAB)
Frequency of dosing: single injection
Dosing volume: 10 ml/kg bw
Control groups:
negative: 1 x vehicle control (10 ml/kg bw olive oil)
positive: 1 x 20 mg/kg bw cyclophosphamide (CPP) for clastogenic effects (10 ml/kg bw), 1 x 0.15 mg/kg bw vincristine (VCR) for aneugenic effcts (10 ml/kg bw)

TEST CONDITIONS
Sampling times: 24 and 48 hrs after the last treatment, samples of bone marrow of the 2 femora were taken and prepared. Preparation of the bone marrow: according to the method of Schmidt (1976 and 1977) and Salamone et al. (1980).

Microscopic evaluation: 2000 polychromatic erythrocytes (PCEs) from each animal of every test group were investigated for micronuclei (MN). The normochromatic erythrocytes (NCEs) were also scored. The ratio of polychromatic to normochromatic erythrocytes was determined.

Clinical observations: after administration of the vehicle, test substance and positive controls, the animals were examined for clinical signs of toxicity.

Criteria for selection of M.T.D.:
In a pretest for determination of the acute i.p. toxicity, deaths were observed at 2,000 mg/kg bw. 1,000 mg/kg bw and 750 mg/kg bw led to evident signs of toxicity and some animals were sacrifced moribund. 500 mg/kg bw were survived by all animals but led to signs of clinical toxicity. There were no distinct differences in the symptoms between males and females. Thus, only males were used for the cytogenetic investigations. Doses of 750, 500 and 250 mg/kg bw were selected for the main test.

Statistical method: U-test according to Mann-Whitney (modified rank test according to Wilcoxon) for differences between control and dose groups.

GLP:
The study was conducted in the GLP Principles of the German Chemicals Act and according to the OECD Principles of Good Laboratory Practice.

REGULATORY GUIDELINES
OECD No. 474 (July 21, 1997)
EEC Directive 2000/32, B. 12 (May 19, 2000)

EVALUATION CRITERIA

The test chemical was considered positive if the following criteria were met:
- A dose-related and significant increase in the number of micronucleated poly-chromatic erythrocytes was observed.
- The proportion of cells containing micronuclei exceeded both the values of the concurrent negative control range and the negative historical control range.

A test substance was considered negative if
- There was no significant increase in the number of micronucleated polychromatic erythrocytes at any dose above concurrent control frequencies.
- The frequencies of cells containing micronuclei were within the historical control range.

Test substance: Purity: 97.8% (area)

Conclusion: Under the experimental conditions chosen, the test substance beta-Ionone did not have any chromosome-damaging (clastogenic) effect, and there were no indications of any impairment of chromosome distribution in the course of mitosis (aneugenic activity) in bone marrow cells in vivo.

Reliability: (1) valid without restriction
GLP guideline study

Flag: Critical study for SIDS endpoint

09-AUG-2004 (137)

5.7 Carcinogenicity

Species: mouse **Sex:** female
Strain: other: ICR Swiss
Route of administration: dermal
Exposure period: 18 weeks
Frequency of treatment: 5 times per week
Post exposure period: no
Doses: 0.25 ml of a 0.04 % solution in aceton (ca. 0.1 mg/animal, ca. 4 mg/kg bw)
Result: negative
Control Group: yes

Method: other
Year: 1971
GLP: no
Test substance: other TS

Result: Beta-Ionone had little or no effect on the incidence of tumors.

Table
Percent of animals with tumors and number of papillomas per surviving mouse

Group	% with tumors	number of papillomas
vehicle	0	0
DMBA	0	0
DMBA+CR	90	12.2
BI+DMBA	0	0
BI+DMBA+CR	90	9.7

Test condition: Groups of 30 freshly shaved ICR Swiss female animals, 55-60 days old, were initiated once with a solution of 0.125 mg

7,12-dimethylbenz[a]anthracene (DMBA) dissolved in 0.25 ml acetone applied to their backs. No additional treatments were given for 3 weeks. Then, 0.25 ml of test material or mixture of 0.006% croton resin (CR) and test material dissolved in acetone was applied to animal skin five times weekly for 18 weeks. Beta-Ionone (BI) was tested at a concentration of 0.04%.
The animals were examined weekly and the number and distribution of tumors were noted. The animals were shaved twice monthly and weighed at monthly intervals during the experiment. The group that received the DMBA and the tumor promotor were the positive control group. One group received DMBA only and one group received acetone only.

Test substance: beta-Ionone, no further data on purity

Reliability: (3) invalid
Unsuitable test system, only one (low) dose tested

15-JUN-2004 (138)

5.8.1 Toxicity to Fertility

Type: other: sub-chronic
Species: rat
Sex: male/female
Strain: Wistar
Route of administration: oral feed
Exposure Period: 3 months
Frequency of treatment: continuously
Doses: 100, 1000, 10000 ppm
Control Group: yes, concurrent vehicle
Result: No effects on reproductive organs or sperm parameters were observed up to the highest tested concentration of 10000 ppm (720 and 801 mg/kg bw/day for males and females).

Method: other: OECD 408
Year: 2004
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Result: TEST SUBSTANCE INTAKE

Dose group	Conc. (ppm)	Intake (mg/kg bw/d) Males	Females
Low	100	7.1	8.2
Mid	1000	71.8	83.0
High	10000	719.6	801.0

The study results concerning ANALYSES, CLINICAL OBSERVATIONS, CLINICAL PATHOLOGY, PATHOLOGY and STATISTICAL METHODS are fully described in chapter 5.4 (Repeated Dose Toxicity). In the following only observations relevant for the endpoint reproductive toxicity are included.

- Estrous cycle determination: No substance-related effects were obtained.

CLINICAL PATHOLOGY

- Sperm analysis: There were no treatment-related effects on

sperm parameters.

PATHOLOGY

- Body weight and reproductive organ weights:
In high dose males, body weight (day 7) and body weight change (day 7 and 14) was statistically significantly decreased.
The weights of the reproductive organs were not influenced by the exposure, except of a significantly increased absolute and relative weight of the testes (+12% and +18%, repectively) and relative weight of epididymides (+11%) in the high dose males (see table 1 below). This is considered to be most likely a result of the decreased mean terminal body weight in these animals as the absolute values for the weight parameters are fully in the range of the historical control data for the same rat strain and the performing laboratory.

- Gross lesions and histopatholgy:
No histological abnormalities in the sex organs were detected and no histological correlates were obtained for the changes in weights of the testes and epididymides. Considering the normal biological variation of these organ weight parameters in connection with the reduced terminal body weight of the high dose animals these observations were not regarded as adverse effects.

In the females, no treatment related effects on sex organs were observed.

Table 1
Absolute and relative testes and epididymides weights

Testes	absolute (g)	relative
Control	3.198	0.948
100 ppm	3.38	0.969
1000 ppm	3.338	0.963
10000 ppm	3.577**	1.114**

Historical (n=20) 2,903 - 3.563 (MW 3.309, SD0.171)
MW = mean value, SD = standard deviation ** p <= 0.01

Epidiymides	absolute(g)	relative
Control	1.071	0.317
100 ppm	1.047	0.301
1000 ppm	1.112	0.321
10000 ppm	1.135	0.352*

Historical (n=19) 0.772 - 1.238 (MW 1.086, SD 0.066)
MW = mean value, SD = standard deviation, * p <= 0.05

Test condition: TEST ORGANISM

Strain: CrlGlxBrlHan:WI (Supplier: Charles River, Germany)
Age at study initiation (day 0): 36 +/- 1 days
Weight at study initiation: about 150 g (males) and about 120 g (females)
Number of animals per group: 10 per dose and sex

ADMINISTRATION / EXPOSURE

- Duration of test/exposure: 90 days
- Test substance purity: 97.8% (HPLC)
- Vehicle: diet (Kliba maintenance diet mouse/rat "GLP")
- Preparation of test formulation: For each concentration, the test substance was weighed out and mixed with a small amount of food in a beaker. Subsequently, a premix was prepared in a mixer by adding an appropriate amount of food followed by mixing. Then corresponding amounts of food, depending on dose group, were added to this premix in order to obtain the desired concentrations. Mixing was carried out for about 10 minutes in a laboratory mixer.

- Stability of test substance in vehicle:
The stability of the test substance in the diet over a period of 49 days at room temperature was proven before the start of the study. Homogeneity and concentration control analyses of the test substance preparations were performed in samples of all concentrations at the start and at the end of the administration period.

The study conditions concerning CLINICAL OBSERVATIONS, CLINICAL PATHOLOGY, PATHOLOGY and STATISTICAL METHODS are fully described in chapter 5.4 (Repeated Dose Toxicity). In the following only examinations relevant for the endpoint reproductive toxicity are included.

- Estrus cycle determination:
Vaginal smears for cycle determination were prepared in the morning and evaluated from day 63 until day 91 of the study. The differentiation was conducted to following stages:

Cycle stage	Appearance in vaginal smear
Diestrous	Leucocytes, few nucleated, epithelial cells
Proestrous	Single leucocytes, many nucleated and few horny epithelial cells
Estrous	Only horny epithelial cells
Metestrous	Leucocytes, some horny epithelial cells and some nucleated epithelial cells

CLINICAL PATHOLOGY

- Sperm parameters
Immediately after necropsy and organ weight determination the right testis and cauda epididymis were taken from all male animals. The following parameters were determined: sperm motility, sperm morphology, sperm head count (cauda epididymis), sperm head count (testis)

PATHOLOGY
- Necropsy
The animals were sacrificed by decapitation under CO2 anesthesia. The exsanguinated animals were necropsied and assessed by gross pathology.

- Organ weights
The following weight parameters from all animals sacrificed at scheduled dates were determined: Anesthetized animals,

testes, epididymides, ovaries, uterus, prostate gland

- Histopathology
Left testis, left epididymis and both ovaries were fixed in Bouin' solution. After fixation, the organs were embedded in paraplast. The following organs were fixed in 4% formaldehyde solution, histopathologically processed and examined by light microscopy:
All gross lesions, oviducts/uterus/vagina, prostate gland, seminal vesicles

STATISTICAL METHODS

- Kruskall-Wallis test:
Pathological weight parameters (if p-value < = 0.05 Wilcoxon test was additionally performed)

- Fishers exact test:
Abnormal sperm > 4%

- Wilcoxon test:
Total spermatids/g testis, total sperm/g cauda epi., % motility

Test substance: beta-Ionone, purity 97.8%
Reliability: (1) valid without restriction
GLP guideline study
Flag: Critical study for SIDS endpoint
29-AUG-2005 (127)

5.8.2 Developmental Toxicity/Teratogenicity

Species: rat **Sex:** female
Strain: Wistar
Route of administration: gavage
Exposure period: day 6 through day 19 post coitum (p.c.)
Frequency of treatment: once daily
Duration of test: until gestation day 20
Doses: 100, 300, 700 and 1000 mg/kg bw
Control Group: yes, concurrent vehicle
NOAEL Maternal Toxity: = 300 mg/kg bw

Method: other: Range finding study
Year: 2004
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Remark: Study was a range finding study for a subsequent developmental toxicity study in rats (BASF project no. 30R0449/02056).
Result: At dose levels of 700 and 1000 mg/kg bw/day, a high mortality rate, numerous adverse clinical observations (abdominal position, unsteady gait, twitching, apathy) as well as markedly reduced feed consumption and body weight gain were observed.
Except salivation, no adverse findings were noted at 300 mg/kg bw/day.

Based on these findings, dosages of 25, 100 and 400 mg/kg bw/day were selected for the main study.
Test condition: Beta-Ionone was administered as a solution in oilve oil

(Ph.Eur/DAB) to 10 presumed pregnant female Wistar rats by gavage at dose levels of 0, 100, 300, 700 and 1000 mg/kg bw/day. Food consumption, body weights and state of health were recorded regularly.

Test substance: beta-Ionone, purity 97.8%

Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, well documented and acceptable for assessment.

09-NOV-2004 (139)

Species: rat **Sex:** female
Strain: Wistar
Route of administration: gavage
Exposure period: day 6 through day 19 post coitum (p.c)
Frequency of treatment: once daily
Duration of test: until gestation day 20
Doses: 25, 100, and 400 mg/kg bw
Control Group: yes, concurrent vehicle
NOAEL Maternal Toxity: = 100 mg/kg bw
NOAEL Teratogenicity: = 400 mg/kg bw
NOAEL Fetotoxicity : = 400 mg/kg bw
LOAEL Maternal Toxicity : = 400 mg/kg bw
Result: no influence on gestational parameters, no adverse signs of developmental toxicity, no indications of teratogenic effects

Method: OECD Guide-line 414 "Teratogenicity"
Year: 2004
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: REGULATORY GUIDELINES

- OECD No. 414 (proposal for updating, January 22, 2001)
- US EPA, Health Effects Test Guidelines; OPTTS 870.3700: Prenatal Developmental Toxicity Study (August 1998)

Result: TEST SUBSTANCE ANALYSES

The stability of the test substance suspensions over a period of 4 days (at 4°C) and the correct concentration of the test substance in the test preparation was demonstrated (always above 90% and below 110% of nominal concentrations).

MATERNAL TOXIC EFFECTS BY DOSE LEVEL

- Mortality and day of death: There were no substance-related or spontaneous mortalities in any of the groups.
- Clinical examinations: Each test group including the controls contained a sufficient number of females with implantation sites at necropsy (20 or more).

Clinical symptoms: All high dose and the majority (22 out of 25) of the mid dose animals showed transient salivation immediately after treatment on one or several days of the treatment period; however, the observed salivation persisted in the respective females only for a few minutes after the actual gavaging had taken place. After cessation of treatment on day 19 p.c., salivation did not occur any longer. The observed temporary salivation of the animals was considered to be substance-induced. It is very likely, that this finding was

induced by bad taste of the test substance or local affection of the upper digestive tract. Salivation itself is not assessed as an adverse or toxic effect.
Additionally, 21 high dose dams showed dark-yellow discolored urine on gestation days 12 - 20 p.c. which is probably related to a chemical reaction of the test substance or its metabolites with the bedding or with components of the air and does not represent a toxicologically relevant finding. Comparable urine findings have been observed in a 4-week range finding study in Wistar rats with dietary administration of the test substance, Project No.: 30S0449/02045 (BASF AG, 2003).
No indications for disturbances of the general behavior, however, occurred in the control and low dose dams.

- Food consumption: The mean food consumption of the high dose dams was statistically significantly reduced (9% below the concurrent control value) at initiation of treatment (days 6 - 8 p.c.). On the following days of the treatment period, however, food consumption of the high dose rats reached or even exceeded control values. The food consumption of the females of low and mid dose dams was unaffected and did not show any statistically significant or biologically relevant differences in comparison to the controls.
The transient reductions in food consumption at 400 mg/kg bw were accompanied by corresponding impairments in body weight gain of these dams at initiation of dosing.

- Body weight data: The mean body weights of the low, mid and high dose rats were substantially similar to the concurrent control values.
A statistically significant impairment in mean body weight gain (about 29% below the concurrent control value) occurred in high dose group on treatment days 8 - 10 p.c.; on the other treatment days (i.e. days 6 - 8 and 10 - 19 p.c.) weight gains of the 400 mg/kg bw females were sometimes below and sometimes above those of the corresponding controls without attaining statistical significance. As the food consumption of these rats was also transiently diminished and the corrected body weight gain was also slightly decreased this was considered to be a substance-related sign of maternal toxicity.
Body weight gains of the dams at 25 and 100 mg/kg bw/day) were similar to those of the concurrent controls.

- Corrected body weight gain (net maternal body weight change): The corrected body weight gains (terminal body weight on day 20 p.c. minus weight of the unopened uterus minus body weight on day 6 p.c.) of the dams of the low and mid dose groups revealed no differences of any biological relevance to the corresponding control group. The net weight change of the 400 mg/kg bw rats, however, was about 17% below the concurrent control value (not statistically significant). As food consumption and body weight gain of this group was also temporarily diminished, the effects on net body weight gain at the top dose are considered to be substance-related, borderline signs of maternal toxicity.

EXAMINATION OF THE DAMS AT TERMINATION

- Uterus weight: The mean gravid uterus weights of the animals

of all test groups were not influenced by the administration of the test substance.

- Liver weight: Absolute and relative mean liver weights were statistically significantly increased at the mid and high dose groups and were about 9 or 29% (absolute) and 8 or 29% (relative) above control values. These weight increases, which are considered to be substance-induced, are indicative of hepatic changes primarily caused by microsomal enzyme induction. Absolute and relative liver weights of the low dose dams, however, were similar to the control values and did not show any toxicologically significant changes.

- Necropsy findings: There occurred no substance-related observations at necropsy in any of the dams of all test groups. Only very few spontaneous findings were recorded for single low and mid dose rats (one hydrometra in low dose female which consequently did not become pregnant, hemorrhagic thymus in one mid dose female). No association to the test compound was assumed for these findings due to their scattered occurrence without any relation to dosing.

- Reproduction data of dams: The conception rate reached 96% in the controls, 92% in the low and the high dose groups, and 100% in the mid dose. There were no substance-related and/or biologically relevant differences between the test groups in the conception rate, in the mean number of corpora lutea and implantation sites, the pre- and the postimplantation losses, and the number of resorptions and viable fetuses (see table below). The pre- and the postimplantation loss values in the 25 and 100 mg/kg groups, however, were above the upper ranges of the historical control values and the mean number of live fetuses/low dose dam was statistically significantly below the concurrent and the historical control value. These differences appeared without any dose-response relationship and thus are not considered to reflect any substance-induced effect. They can well be explained by the fact that one low dose dam and two mid dose dams resorbed all of their implants and thus had no viable fetuses (the same was also observed for one control dam). Moreover, one low dose, which had 7 implantation sites, resorbed 6 implants and had only one live fetus at terminal sacrifice. If these 5 rats are excluded from the calculation of the means, pre- and postimplantation loss values as well as the mean number of live fetuses/dam fit well into the historical control ranges with one unimportant exception (preimplantation loss value at 25 mg/kg bw/day).

Table: Pre- and postimplantation loss (PRI and POI) values and mean number of live fetuses per dam

Dose	Control	Low	Mid dose	High	Historical control range
PRI	6.0	14.4	12.7	4.7	8.7 (3.5-12.2)
Corrected	4.8	12.9*	8.7	4.7	
POI	9.9	14.8	15.2	6.2	6.7 (3.7-11.3)
Corrected	6.0	7.3	7.9	6.2	

Live fetuses per dam	8.7	7.4*	8.2	8.6	8.8 (7.9-9.8)
Corrected	8.7	7.7	8.2	8.6	

Corrected: exclusion of 5 dams, * P < = 0.05

EXAMINATION OF FETUSES

- Sex distribution of fetuses: The sex distribution of the fetuses in all test groups was comparable with that of the control fetuses.
- Weight of placentae: The mean placental weights in the substance-treated groups did not show any differences with toxicological relevance. The statistically significant increase of the mean placental weight of the high dose male fetuses (0.45g versus 0.41g in the control group; p <= 0.05) is not considered to demonstrate an adverse finding and the respective value was fully within the historical control range (0.32g-0.58g).
- Weight of fetuses: The mean fetal body weights in all test groups were not influenced by the test substance administration and were very similar to or even identical with concurrent control values.
- Fetal external, soft tissue and skeletal observations: The scattered occurrence of the few observed external, soft tissue and skeletal malformations in single fetuses of the controls, the low and mid dose group without a consistent pattern, without any dose-response relationship and/or at incidences, which are similar to historical control rates did not suggest any substance-induced origin of these findings. Most of the observed malformations were limited to one multiply malformed mid dose fetus. The external examination of this fetus revealed gastroschisis, anal atresia, malrotated left hindlimb, and a thread-like tail. During its skeletal evaluation additional malformations like severely malformed sternum and absent vertebrae were recorded. The other malformations that occurred were anasarca (in one control fetus), situs inversus (in one low dose fetus), and cleft sternum (in another control fetus).

If all different types of malformations are summarized, in total 2 of the 200 examined control fetuses [= 1.0%] in 2 out of 23 litters [= 8.7%], one of the 163 examined low dose fetuses [= 0.6%] in one out of 22 litters [= 4.5%], one of the 189 examined mid dose fetuses [= 0.5%] in one out of 23 litters [= 4.3%] and none of the 197 examined high dose fetuses (from 23 litters) showed malformations. The mean percentages of affected fetuses/litter with total malformations amounted to 0.8, 0.5, 0.6, and 0.0% at 0; 25; 100 or 400 mg/kg bw/day respectively. These low, non dose-related incidences did not suggest any relationship to the test substance.

External variations did not occur in any of the fetuses in this study. Soft tissue variations, exclusively in the form of dilated renal pelvis and/or ureters, and a broad range of skeletal variations occurred in all test groups including the controls. All fetal and litter incidences for these variations and the corresponding mean percentages of affected fetuses/litter did not show a clear relation to dosing, were

not considered to be of any toxicological relevance and/or could be found at a comparable frequency in the historical control data. This statement included the statistically significantly increased occurrence of three variations (i.e. supraoccipital holes, bipartite ossification of thoracic centrum [with dumbbell-shaped cartilage of centrum] and supernumerary 14th rib [without cartilage]) at the mid dose group.

If all variations were summarized, in total 105 of the 200 examined control fetuses [= 53%] in all 23 litters [= 100%], 88 of the 163 examined low dose fetuses [= 54%] in all 22 litters [= 100%], 99 of the 189 examined mid dose fetuses [= 52%] in all 23 litters [= 100%] and 97 of the 197 examined high dose fetuses [= 49%] in all 23 litters [= 100%] showed variations. The mean percentages of affected fetuses/litter with total variations amounted to 52.8, 56.2, 52.9, and 50.8% at 0; 25, 100 or 400 mg/kg bw/day respectively. These incidences did not suggest a treatment-relationship, but reflect the usual biological variation inherent in the strain of rats used for this experiment.

A spontaneous origin was also assumed for the few unclassified cartilage observations which were recorded for several fetuses of all test groups. Distribution and type of these findings do not suggest any relation to treatment as the mean percentages of affected fetuses/litter with these findings amounted to 17.0, 18.5, 15.4, and 13.6% at 0; 25; 100 or 400 mg/kg bw/day, respectively.

Test condition: TEST ORGANISMS
Strain: Sexually mature, virgin Wistar rats (CrlGlxBrlHan:WI) supplied by Charles River Laboratories (Germany)
Number: 25 female animals per group
Age at study initiation: about 70-84 days
Weight at study initiation: 148.7-183.6 g

GLP
The study was conducted in accordance with the OECD Principles of Good Laboratory Practice and with the GLP regulations of the German "Chemikaliengesetz" (Chemicals Act).

ADMINISTRATION / EXPOSURE
- Duration of test/exposure: from implanation to one day prior to the expected day of parturition (day 6 to day 19 post conception). On day 20 p.c., all surviving females were sacrificed.
- Treatment: orally by gavage always at approx. the same time of day (in the morning)
- Control group and treatment: gavage application of 5 ml/kg bw olive oil
- Vehicle: olive oil (Ph.Eur./DAB)
- Test substance preparation: At the beginning of the administration period and thereafter at intervals which took into account the analytical results of the stability verification. For the preparation of the solutions an appropriate amount of the test substance was weighed depending on the dose group, in a graduated beaker, topped up with olive oil, and subsequently thoroughly mixed using a magnetic stirrer.

- Concentration in vehicle: 500, 2000 and 8000 mg/100 ml
- Total volume applied: 5 ml/kg bw
- Doses: 25, 100, 400 mg/kg bw (dosages were selected due to the results of a range finding study; BASF project no. 10R0449/02050)
- Analyses: check of stability and concentration control was performed by HPLC. Since the test substance were true solutions, investigations concerning homogeneity were not necessary.

MATING PROCEDURES
The animals were mated by the breeder ("time-mated") and supplied on day 0 post coitum (= detection of vaginal plug / sperm). The animals arrived on the same day (i.e. day 0 p.c.) at the experimental laboratory. The following day was designed "day 1" post coitum (p.c.). Animals were assigned to the test groups by taken random selection.

PARAMETERS ASSESSED DURING STUDY
- Mortality: A check was made twice a day on working days or once a day (Saturday, Sunday or on public holidays) (days 0 - 20 p.c.).
- Clinical symptoms: The animals were examined for clinical symptoms at least once a day, or more often when clinical signs of toxicity were elicited (days 0 - 20 p.c.).
- Body weight gain: All animals were weighed on days 0, 1, 3, 6, 8, 10, 13, 15, 17, 19 and 20 p.c.. The body weight change of the animals was calculated from these results.
- Food consumption: With the exception of day 0, the consumption of food was determined on the same days as was body weight.
- Corrected body weight gain (net maternal body weight change) Furthermore, the corrected body weight gain was calculated after terminal sacrifice (terminal body weight on day 20 p.c. minus weight of the unopened uterus minus body weight on day 6 p.c.).
- Examination of uterine content: Gravid uterine weight, number of corpora lutea, number and distribution of implantation sites classified as live fetuses, dead implantations, early resorptions, late resorptions and dead fetuses. Calculations of conception rate and pre- and postimplantation losses were carried out.
- Examination of fetuses after dissection from the uterus: Litter size, fetal weight, sex ratio, grossly visible/external/soft tissue/skeletal abnormalities. The viability of the fetuses and the condition of the placentae, the umbilical cords, the fetal membranes and fluids were examined. Individual placental weights were recorded. After these examinations, approximately one half of the fetuses per dam were eviscerated, skinned and placed in ethyl alcohol, the other half was placed in BOUIN's solution for fixation and further evaluation.
- Soft tissue examination of the fetuses: The fetuses fixed in BOUIN's solution were examined for any visceral findings according to the method of BARROW and TAYLOR (1969).
- Skeletal examination of the fetuses
The skeletons of the fetuses fixed in ethyl alcohol were stained according to a modified method of KIMMEL and TRAMMELL (1981). Thereafter, the skeletons of these fetuses were examined under a stereomicroscope. After this examination the

stained fetal skeletons were retained individually.
- Evaluation criteria for assessing fetuses: the glossary of WISE et al. (1997) was used as much as possible to describe findings in fetal morphology. Classification of these findings was based on the terms and definitions proposed by CHAHOUD et al. (Chahoud et al., 1999; Solecki et al., 2001; Solecki et al., 2003).

STATISTICAL METHODS
Statistical analyses were performed according to following schedule:
- DUNNETT-test (two-sided): Food consumption, body weight, body weight change, corrected body weight gain, carcass weight, weight of unopened uterus, number of corpora lutea, number of implantations, number of resorptions, number of live fetuses, proportions of preimplantation loss, proportions of postimplantation loss, proportions of resorptions, proportion of live fetuses in each litter, litter mean fetal body weight, litter mean placental weight
- FISHER'S EXACT test (one-sided): Female mortality, females pregnant at terminal sacrifice, number of litters with fetal findings
- WILCOXON-test (one-sided): Proportions of fetuses with malformations, variations and/or unclassified observations in each litter
- KRUSKAL-WALLIS-test (two-sided): Liver weights

HISTORICAL CONTROL DATA
The historical control data used for interpretation of findings refer to the same test facility, the same rat strain and supplier of the animals and cover a period of about 24 months (June 2001 - June 2003, 15 studies).

Test substance: beta-Ionone, purity 97.8%

Conclusion: Based on the results of this prenatal developmental toxicity study, beta-Ionone had no influence on gestational parameters and induced no adverse signs of developmental toxicity up to and including the high dose level (400 mg/kg bw/day); especially, no indications of teratogenic effects occurred which could be causally related to the test substance administration.

Based on these results, the no observed adverse effect level (NOAEL) for maternal toxicity is 100 mg/kg bw/day. The NOAEL for prenatal developmental toxicity could be fixed at 400 mg/kg bw/day.

Reliability: (1) valid without restriction
GLP guideline study

Flag: Critical study for SIDS endpoint

09-NOV-2004 (140)

Species: rat **Sex:** female
Strain: Wistar
Route of administration: gavage
Frequency of treatment: single application on day 11 of pregnancy
Duration of test: until day 21 of pregnancy
Doses: 250, 500, 750 and 1000 mg/kg bw
Control Group: yes, concurrent vehicle
NOAEL Maternal Toxity: = 1000 mg/kg bw

Method: other

Year: 2002
GLP: no data
Test substance: other TS

Remark: The study was performed to investigate whether inhibition of CYP2B1 reactions by beta-Ionone (BI) could lead to an attenuation of cyclophosphamide (CP)-induced embryotoxicity in the rat.

Result: MATERNAL TOXICITY
No overt signs of maternal toxicity and no dose related reduction of pregnancy weight, after deduction of gravid uterus weight at term were noted in dams treated with beta-Ionone alone or in combination with CP.

EXAMINATION OF DAMS AN FETUSES
Beta-Ionone treatment:
At the high dose, the ratio of resorptions per implantations and the percentage of resorptions per implantations per litter were significantly increased (95% and 100% versus 13.1% and 9.2% in controls). In the same group, the ratio of live fetuses per implantations (5.0% versus 86.9%) and the number of live fetuses per litter was decreased (0.6 versus 10.5% in controls). In the lower dose groups (750, 500 and 250 mg/kg bw) no treatment related changes were observed.
No externally-visible anomaly was noted in fetuses treated orally with beta-Ionone wheras all fetuses treated with CP alone exhibited skull anomalies the severity of which varied from slight to severe.

CP treatment:
A single dose of CP at day 11 of pregnancy caused a high frequency (about 60%) of embryo deaths, however the CP-induced embryolethality was clearly reduced by previous treatment with single oral doses of beta-Ionone ranging from 250-750 mg/kg bw. Furthermore, beta-Ionone administered previously to CP induced a statistically significant decrease in the proportion of fetuses showing anomalies, in particular when skull defects were scored for severity.

The authors concluded that beta-Ionone antagonizes the embryolethal and embryotoxic effect of CP at doses which were per se not embroytoxic.

Test condition: TEST ORGANISM AND ADMINISTRATION
Male and female Wistar rats supplied by the Center for Breeding of Laboratory Animals of the Oswaldo Cruz Foundation weighing approximately 220g (range: 200-250g) were used.
Mating was performed by placing 2 females into the cage of 1 male for 2 hrs and confirmed by the presence of sperm in the vaginal smear (day 0 of pregnancy).
The rats were treated by gavage with a single dose of beta-Ionone (250, 500, 750 and 1000 mg/kg bw) or with the vehicle corn oil (3.75 g/kg bw). The number of pregnant females was 22, 12, 9, 12 and 10 for the controls, 250, 500, 750 and 1000 mg/kg bw, respectively.
45 minutes after having received the test substance dams were treated with a subcutaneous injection of cyclophosphamide (CP) (7.5 mg/kg bw dissolved in saline) in the dorsal area of the neck.

EXAMINATIONS

The rats were weighed on days 0, 11 and 21 of pregnancy. On day 21 all dams were killed. The gravid uterus was weighed with its contents and the number of implantation sites, living and dead fetuses and resorptions was recorded.
The living fetuses were weighed, examined for externally visible anomalies under a stereomicroscope and fixed in a 5% formalin solution. The externally visible skull defects were scored for severity using a grading scale.

STATISTICS
Data were analysed by one-way analysis (ANOVA) followed by the Scheffe's test for multiple comparisons, or alternatively by the Kruskal-Wallis test followed by the Mann-Whitney U-test whenever the variable did not fit a normal distribution.

Test substance: beta-Ionone, purity 95%

Conclusion: The results of the study support the hypothesis that beta-Ionone modulates the clearance and metabolic activation of cyclophosphamide and other xenobiotics which are substrates of isoenzymes belonging to the CYP2B subfamily. The data suggest that concomitant exposure of beta-Ionone slows the conversion into its embyrolethal and teratogenic metabolites.

Reliability: (3) invalid
Significant methodological deficiencies:
- only single application (OECD 414: from implantation to day prior to scheduled caesarean section)
- to few animals with implantation sites at necropsy (9-12 in substance treated groups; OECD 414: approximately 20)
- no soft tissue alterations were investigated

15-JUN-2004 (141)

Species: hamster **Sex:** female
Strain: other: Golden Syrian
Route of administration: gavage
Exposure period: single intubation on day 8 of pregnancy
Frequency of treatment: single dose
Duration of test: up to day 14 of pregnancy
Doses: 48; 240; 480 mg/kg bw/day
Control Group: yes, concurrent vehicle
NOAEL Maternal Toxity: 480 mg/kg bw
NOAEL Teratogenicity: 480 mg/kg bw

Method: other: Willhite (1986). Toxicol. Appl. Pharmacol. 83, 563-575
Year: 1986
GLP: no data
Test substance: other TS

Result: Treatment with up to 480 mg/kg bw beta-Ionone failed to alter significantly the incidence of abnormal litters or mean litter fetal body weight. No maternal toxicity reported.

Test condition: Timed pregnant LAK:LVG(SYR) hamsters, weighing 99-183 g, were used (6-14 animals per group). Test material was dissolved in acetone and solubilized in polyoxyethylene sorbitan monolaurate. Final acetone concentration was 5%. Animals received a single dose of test material solution at 10 am on day 8 of pregnancy. Animals were sacrificed on day 14 of pregnancy. Pregnant uteri were collected after laparatomy. The numbers of resorption and dead fetuses were recorded. Live fetuses were weighed and one-third of each litter was fixed in Bouin's fluid and subsequently sectioned in the mid-sagittal plane. Two-thirds of each litter were processed for skeletal

examination. Abnormal litters were those containing one or more malformed fetuses or three or more resorbed implantation sites.
Test substance: Beta-Ionone, purity 98% and 99%
Reliability: (3) invalid
Significant methodological deficiencies (only single application, to few animal per dose group)
15-JUN-2004 (142)

Species: rat **Sex:** female
Strain: Wistar
Route of administration: oral unspecified
Exposure period: 4 days from day 9 to day 12 of pregnancy
Frequency of treatment: no data
Duration of test: no data
Doses: 60; 134; 670 mg/kg bw/day
Control Group: other: physiological saline

Method: other
Year: 1968
GLP: no
Test substance: other TS

Remark: Original article in Japanese language
Result: Body weight of mother rats
As for the increase in body weight of mother rats, there was almost no difference relative to the control group. Number of pregnant females not reported.

Status of fetuses
- Fetal deaths were found in both groups of ß-ionone in contrast to the control group (percentage of survived fetuses: 100%, 96.5%, 91% and 27.2% for controls, low, mid and high dose animals, resp.).
- The number of resorptions was increased (0%, 2.8%, 5.7% and 68.4% for controls, low, mid and high dose animals, resp.).
- The percentage of abnormal fetuses were 0% (controls), 0% (low dose), 0% (mid dose) and 2.7% (high dose; analatresia and ecaudate).
- The percentage of fetuses with retardation of ossification of the occipital bone and of the sternebrae was increased compared to controls which was, however, independent from dose.

Test condition: Wistar albino rats were used. The total number of female rats treated were 17 (controls), 11 (60 mg/kg bw), 10 (134 mg/kg bw) and 11 (670 mg/kg bw).
It is indicated that breeding environment, experimental feed, pregnancy determination, observation period and skeletal preparation method were the same as those of the previous report (no reference given).
Test substance: beta-Ionone, no further data on purity
Reliability: (3) invalid
Significant methodological deficiencies. Too few animals used (10-11 treated female rats instead of >= 16 pregnant animals according to OECD 414), no details on protocol provided, no substance specification given, documentation of results insufficient).
24-AUG-2005 (143) (144)

5.8.3 Toxicity to Reproduction, Other Studies

5.9 Specific Investigations

5.10 Exposure Experience

Remark: A patch test using ionone (mixture of the alpha- and the beta-isomer) full strength for 24 hours produced no reactions in eleven subjects.
Reliability: (4) not assignable
SECONDARY LITERATURE
Flag: non confidential, Critical study for SIDS endpoint
05-SEP-2005 (145)

Remark: A maximization test was carried out on 25 volunteers. Ionone was tested at a concentration of 8 % in pet. and produced no sensitization reactions.
Attached doc.: 38393.pdf
Reliability: (4) not assignable
DOCUMENTATION INSUFFICIENT FOR ASSESSMENT
Flag: non confidential, Critical study for SIDS endpoint
10-NOV-2004 (146)

Remark: Patch tests with three ionones (alpha ionone, methyl ionone and extra pure ionone, without further psecification) were performed on 20 patients with different dermatoses. The ionones were used undiluted. The three ionones elicited negative reactions in all subjects, except for a doubtful reaction in 2 persons. These reactions were erythematous and disappeared within forty-eight hours.
Attached doc.: 38423.pdf
Reliability: (4) not assignable
DOCUMENTATION INSUFFICIENT FOR ASSESSMENT
Flag: non confidential, Critical study for SIDS endpoint
10-NOV-2004 (147)

5.11 Additional Remarks

Type: Behaviour

Remark: The effects of test material on the motility of mice after inhalation was studied.
Result: Positive effects were observed. The motility of the mice was increased (+14.20% when compared to controls). When the animals were pretreated with Caffeine (0.5 ml 0.1% solution; i.p.) the motility was decreased (-27.97%).
Test condition: Groups of four female outbred Swiss mice with mean body weights of 28.5 grams were used. Both 4-6 week old and 6 month old mice were treated. Air was passed into the cage through a glass tube containing 1.5 ml test material. Total test material volume was 20-50 mg. Exposure time was one hour. Test concentration in the atmosphere was not mentioned. Effects of test material on motility were observed and are expressed in

percent decrease or increase as compared to untreated control animals. Motility of untreated control animals = 100%.

Test substance: beta-Ionone, no further data on purity

Reliability: (3) invalid
Unsuitable test system

09-NOV-2004 (148)

Type: Behaviour

Remark: Several dicyclic and monocycclic terpenons (including Ionone) were studied for their central stimulating effects in various test systems.

Result: AROUSAL ACTION
The sleeping time for Ionone was determined to be 722 +/- 91 seconds versus 2443 +/-36 (hexobarbital sodium) and 2559 (hexobarbital sodium and sesame oil). Ionone was among the tested compounds the most active substance.

EFFECT ON PENTOBARBITAL-DEPRESSED RESPIRATION
Ionone produced a small but consistent increase in the depth of respiration at the applied dose level.

COORDINATED MOVEMENT
Ionone caused a dose dependent increase in running activity compared to the vehicle controls.

TOTAL MOVEMENT
From all tested compounds only d-amphetamine induced a marked increase in actvity.

Test condition: AROUSAL ACTION
Male albino mice (10 per group) weighing from 18-25 g were administered by i.p. injection Hexobarbital sodium at a dose of 500 mg/kg bw. Five minutes later 500 mg/kg bw of Ionone (using sesame oil as a vehicle) was subcutaneously injected. Control groups received sesame oil, hexobarbital or hexobarbiotal followed by sesame oil. The end point was the sleeping time (determined when the mouse was sufficiently coordinated to climb out a box into which air was blown).

EFFECT ON PENTOBARBITAL-DEPRESSED RESPIRATION
Rats weighing from 200-300 g were anesthetized with ether, the trachea cannulated and the cannula attached to sensitive onlet and outlet valves (4 animals per group).
Respiration was depressed by i.p. administration of 35 mg/kg bw of Pentobarbital sodium (further 5 mg/kg bw increments were optionally administered to uniformly decrease respiration). When the desired decrease had been achieved, 500 mg/kg bw of the tepenenone was injected i.p.. An aqueous solution of d-amphetamine sulfate (20 mg/kg bw; i.p.) was used for the purpose of comparison.

COORDINATED MOVEMENT
The effect of the test substances on running or walking activity was determined with a vertically revolving activity drum. 4 black-hooded male rats (200-350 g) were used for each dose level. Ionone in sesame oil, the vehicle and positive control substances (picrotoxin, strychnine and d-amphetamine) were administered i.p. at doses of 50, 100, 200 and 400 mg/kg bw.

TOTAL MOVEMENT
The spring-suspended cage of Schulte (1939) was used to measure total movement. The apparatus records all motion including tremor and convulsions as well as voluntary activity. The experimental procedure was similar as in the preceding study.

Test substance: Mixture of alpha-Ionone and beta-Ionone; no further data

Reliability: (3) invalid
Significant methodological deficiencies

02-JUN-2004 (149)

Type: Biochemical or cellular interactions

Remark: Protein kinase C (PKC) is a ubiquitous enzyme linked to transmembrane signal transduction. The effects of retinoids (including beta-Ionone) on T-cell derived PKC was studied. Furthermore, the regulatory effects of retinoids on PKC activity were compared with those of common membrane phospholipids.

Beta-Ionone did not affect PKC activity tested at a concentration range of 10E-7 to 10E-3 M.

Test substance: beta-Ionone, no further data on purity

Reliability: (2) valid with restrictions
Scientifically acceptable method

14-MAY-2004 (150)

Type: Biochemical or cellular interactions

Remark: Chicks were fed a diet containing test material (0.5 mmol/kg). No additional information provided.

Results
Body weight changes (+10%), significantly lower hepatic reductase activities (-47%) and serum cholesterol levels (-16%). Body weight gain was increased (+10%).

Test substance: beta-Ionone, indication of purity is missing

Reliability: (4) not assignable
Abstract

15-JUN-2004 (151)

Type: Biochemical or cellular interactions

Remark: The objective of the study was to determine the inductive effect of beta-Ionone on liver cytochrome P450 2B1 in Sprague Dawley rats and to characterize the regulatory mechanism of induction.

Result: ENZYME INDUCTION
Liver microsomal PROD activity and P450 2B1 levels were increased in a dose dependent manner at 300 mg/kg bw dose and above. The effects were significantly greater in males than females.

Liver microsomal P450 2B1 mRNAs were elevated at 600 mg/kg bw beginning 6 hrs after dosing in males and 12 hrs after dosing in females.

Test condition: A single dose of test material was administered by s.c. injection to 4 Sprague-Dawley rats (6-8 weeks of age) per sex per sacrifice interval. Doses applied were 300, 600 and 1200 mg/kg bw. The vehicle was corn oil. Sacrifice was 72 or 48 hours after dosing. The observations were microsomal pentoxyresorufin O-dealkylase (PROD) activity and cytochrome P450 2B1 protein levels. Additional rats received the mid dose only for observation of mRNA levels at 6, 12, 24 and 48 hours after dosing.
Test substance: beta-Ionone, no further data on purity
Conclusion: According to the authors, the results indicate that the induction of cytochrome P450 2B1 by beta-Ionone might be regulated by the accumulation of mRNAs.
Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, well documented and acceptable for assessment

15-JUN-2004 (152)

Type: Biochemical or cellular interactions

Remark: The effect of the simultaneous addition of various test materials on L5178Y cell growth inhibition was studied.
Result: The IC50 (50% inhibiting concentration) against mouse leukemia L5178Y cells was 3.0 10-5 M.
Strong synergistic effects were produced with beta-Ionone in combination with trans-beta-carotene, trans-retinol, trans-retinal palmitate, phytol, farnesol, squalene, alpha-terpinene, or dl-alpha-tocopherol. Weak or no synergistic effects were produced with beta-Ionone in combination with trans-retinal, trans-retinoic acid, bixin, abscisic acid, farnesene, nerol, citral, dipentene, isophorone, nerolidol, or phylloquinone.
Test condition: L5178Y cells were cultured in RPMI 1640 medium supplemented with 10% calf serum, and kept in an incubator containing 5% CO2 in humidified air at 37°C. The test materials in DMSO were added to a 96-well tissue culture microtitre plate and following their addition, the L5178Y cells were incubated for 5 days. The controls were cultures that DMSO alone had been added to. A hemocytometer was used to count the number of viable cells, and viability was monitored by means of trypan blue exclusion. A total of 20 materials were used at the same dose level, and all possible paired combinations of the materials were tested. The mean cell number of the viable cells in the test samples and the control samples were determined.
The synergistic effects were classified as strong, weak or negligible
Test substance: beta-Ionone, no further data on purity
Reliability: (2) valid with restrictions
Scientifically acceptable method

09-AUG-2004 (153)

Type: Biochemical or cellular interactions

Remark: Inductive effects of cytochrome P450 2B1 by alpha- and beta-Ionone were characterized in individual liver lobes of male Sprague Dawley rats.
Result: 100 mg/kg bw

EROD activity was increased in all liver lobes except the caudate and right posterior lobes; MROD activity was increased in all liver lobes except the caudate, right posterior and right anterior lobes; PROD activity was increased in all liver lobes except the right posterior lobe; and BROD activity was increased in all liver lobes except the caudate lobe.

300 mg/kg bw
EROD, MROD and BROD activity was increased in all liver lobes. PROD activity was significantly ($p < 0.05$ and 0.01) increased in all the lobes, but only in the right posterior lobe was the increase not significant.
600 mg/kg bw
EROD activity was increased in all liver lobes except the left median lobe; MROD activity was increased in all liver lobes except the right anterior lobe; and BROD activity was increased in all the liver lobes. PROD activity was significantly ($p < 0.05$ and 0.01) increased in all the lobes, but only in the right posterior lobe was the increase not significant.

Test condition: Male Sprague Dawley rats were administered oral doses of 100, 300 and 600 mg/kg bw of the test material in corn oil for 24 hours. In each lobe of the livers of the treated and control rats (caudate, right posterior, right anterior, right median, left median and left), the activity of ethoxyresorufin O-deethylase (EROD), methoxyresorufin O-deethylase (MROD), pentoxyresorufin O-deethylase (PROD) and benzyloxyresorufin O-deethylase (BROD) was measured.

Test substance: alpha-Ionone and beta-Ionone; no further data on purity

Conclusion: According to the authors, the results indicate that the different induction of P450s susceptibilities of rat liver lobes to certain hepatotoxicants which require metabolic activation for their toxicity and that alpha- and beta-Ionone may be useful model inducers of P450 2B1 in studying the toxic mechanism of certain toxicants which may require the metabolic activation by P450.

Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, well documented and acceptable for assessment

17-MAY-2004 (154)

Type: Biochemical or cellular interactions

Remark: The possible role of metabolic activation of cytochrome P450 (P450) by beta-Ionone in thioacetamide-induced hepatotoxicity was investigated in male BALB/c mice.

Result: Pretreatment with beta-Ionone significantly potentiated the elevation of ALT and AST activities induced by thioacetamide, particularly with 200 mg/kg bw thioacetamide. Also potentiated was thioacetamide-induced hepatic lesions such as pyknosis, vacuolation, swelling and infiltration of polymorphonuclear cells. Beta-Ionone alone did not affect the actvities of ALT and AST.

The inductive effects of beta-ionone on P450-associated monooxygenase actvities were as follows:

EROD, PROD, APDM and ERDM activities were significantly ($p < 0.05$) induced by the test material, as compared to the

untreated controls. The induction was not in a dose-dependent manner. PNPH activity was also induced, but not significantly.

Test condition: Specific pathogen-free male BALB/c mice were obtained and allowed to acclimate for at least 1 week. The mice at 8 - 10 weeks, weighing 22 - 26.6g, were subcutaneously pretreated with beta-Ionone at 600 mg/kg bw in corn oil, 72 and 48 hours prior to an intraperitoneal administration of either 100 or 200 mg/kg bw of thioacetamide.
The livers were removed and liver microsomes prepared for the assay of ethoxyresorufin O-deethylase (EROD), pentoxyresorufin O-dependentylase (PROD), benzyloxyresorufin O-debenzylase (BROD), p-nitrophenol hydroxylase (PNPH), aminopyrine N-demethylase (APDM), and erythromycin N-demethylase (ERDM) activity.
The activities of serum alanine aminotransferase (ALT) and serum aspartate aminotransferase (AST) were assayed automatically using the Clinical Chemistry Analyzer Shimadzu.
Histopathological examination of the livers was also conducted.

Test substance: beta-Ionone, no further data on purity

Conclusion: Because the pretreatment with beta-Ionone was not hepatotoxic at the dose inducing P450s, their present results suggest that beta-Ionone might be a useful model inducer of P450 enzyme(s) in studying toxic mechanism of certain chemicals which require metabolic activation by P450s in mice.

Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, well documented and acceptable for assessment

10-NOV-2004 (155)

Type: Biochemical or cellular interactions

Remark: The authors recently reported that beta-Ionone induces cytochrome P450 (P450) 2B1 in rats. Effects of beta-Ionone on the expression of other P450-isozymes and NADPH-P450 reductase were further investigated in rats.

Result: Administration of beta-Ionone induced the liver microsomal activities of P450-associated enzymes and NADPH-P450 reductase.
Contents of P450 1A1/2, 2C and NADPH-P450 reductase proteins were increased. The level of P450 1A1/2 and 2C induced by beta-Ionone was also higher in male rats than in female rats.
Reverse transcriptase-polymerase chain reactions showed additionally that P450 2B1 and 2B2 mRNAs, P450 1A2, 2C6 and NADPH-P450 reductase were increased.

Test condition: A single dose of test material was administered by s.c. injection to 4 Sprague-Dawley rats (6-8 weeks of age) per sex per sacrifice interval. Doses applied were 300, 600 and 1200 mg/kg bw. The vehicle was corn oil. Sacrifice was 72 or 48 hours after dosing. The observations were microsomal Ethoxyresorufin O-dealkylase (EROD) activity and cytochrome P450 2B1 protein levels. Additional rats received the mid dose only for observation of mRNA levels at 6, 12, 24 and 48 hours after dosing.

For Western immunoblotting analyses, microsomal protein (10 µg/well) were resolved by 10% SDS-PAGE and were transferred to nitrocellulose filters. The filters were incubated with 2.5% non-fat dry milk for 30 min to block the non-specific binding, and then were incubated with rabbit polyclonal antibodies

against rat P450 1A1/2, 2C, and 3A, and NADPH-P450 reductase, resp, followed by alkaline phosphatase-conjugated goat anti-rabbit IgG antibody. The primary antibody against each isozyme was prepared. For immunostaining, the nitrocellulose filters were developed with a mixture of 5-bromo-4-chloro-3-indolyl phosphate, nitroblue tetrazolium and 0.1 M Tris buffer (1:1:10) under an instruction by the manufacturer.
Reverse transcriptase-polymerase chain reactions were performed to measure P450 1A2, 2B1, 2B2 2C6 and NADPH-P450 reductase expression.

Test substance: beta-Ionone, no further data on purity

Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, well documented and acceptable for assessment

17-MAY-2004 (156)

Type: Biochemical or cellular interactions

Remark: The objective of the study was to examine the effects of CYP inducers (including beta-Ionone) on cocaine-induced heptatoxicity and to elucidate CYP isozyme(s) responsible for cocaine bioactivation in female mice

Result: Effect on cocaine-induced heptatotoxicity:
Pretreatment with beta-Ionone resulted in elevation of plasma AAT, i.e. induced hepatotoxicity of cocaine. The necrotic lesions were found in the periportal region.

Changes of hepatic monooxygenase activities:
beta-Ionone induced CYP1A (2.5 times), 2A (1.5 times) and 2B (twice).
Changes in hepatic and plasma cocaine esterase activities:
There were no significant effects.

CYP-isozymes specific for cocaine-N-demethylation:
Was induced by 1.2 after treatment with beta-Ionone.

Test condition: Administration:
Female ICR mice (7 weeks old) were injected beta-Ionone two times s.c. at a dose of 600 mg/kg bw in corn oil to induced certain CYP enzymes. The control mice received an equal volume of the vehicle.
Measurement of cocaine-induced heptatoxicity:
The mice were injected i.p. with cocaine at a dose of 35-45 mg/kg bw and the diet then withdrawn 52 hrs after the second injection of beta-Ionone.
The mice were bled by cardiac puncture under ether anaesthesia 16-17 hrs after cocaine administration. Alanine aminotransferase (AAT) activity was assayed using Transaminase CII test WAKO. Liver tissues were obtained and prepared for histological examination.
Measurement of microsomal monooxygenase activity and heptatic microsomal and plasma cocaine esterase activity:
The mice were sacrified 48 hrs after the second injection of beta-Ionone. Livers were homogenized and hepatic microsomal monooxygenase activities were measured (N-demethylation of cocaine, erythromycin = CYP3A, or dimethylnitrosamine = CYP2E, 7-pentoxyresorufin O-dealkylase = CYP2B and 7-Ethoxyresorufin O-deethylase = CYP1A1 and coumarine 7-hydroxylation = CYP2A). Additionally hepatic microsomal and plasma cocaine exterase

activity was determined.
Test substance: beta-Ionone, indication of purity is missing
Conclusion: According to the authors, the results of the study suggest that cocaine-induced hepatotoxicity in female mice was mediated in part by CYP 2A, participating in cocaine N-demthylation.
Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, well documented and acceptable for assessment

15-JUN-2004 (157)

Type: Biochemical or cellular interactions

Remark: The effects of alpha- and beta-Ionone on liver P450 1A and 2B expression in rats were investigated after subcutaneous injection. Western immunoblotting analysis and a reverse transcriptase-plymerase chain reaction were used.
Result: Subcutaneous administration of alpha- and beta-Ionone induced the liver microsomal P450 1A and 2B proteins. P450 2B1 induction was associated with the accumlation of its corresponding mRNA. Induction by beta-Ionone was much higher than that by alpha-Ionone in both the mRNA and protein levels. When the route of administration was compared, P450 2B was induced more strongly after oral administration compared to that after subcutaneous injection.
A single oral dose of 100, 300 and 600 mg/kg bw of alpha- and beta-Ionone for 24 hrs induced P450 3B1-selective pentoxyresorufin O-depentylase activity comparably in a dose dependent manner. In addition, alpha- and beta-Ionone induced the P450 1A and 2B proteins.
Test condition: Administration:
Male Sprague Dawley rats (5-6 weeks old) were treated s.c. with the Ionones in corn oil at 100, 300 and 600 mg/kg bw 72 and 48 hrs prior to sacrifice. Microsomes were prepared from livers.
To examine mRNA expression, the male rats were treated with 100, 300 and 600 mg/kg bw s.c. once for 6 hrs.
For assaying the P450-associated monooxygenase activities the rats were treated orally with 100, 300 and 600 mg/kg bw for 24 hrs.
Ethoxyresorufin O-deethylase (EROD) and pentoxyresorufin O-depentylase (PROD actvities were measured.
Microsomal proteins were determined using Western immunoblotting with rabbit polyclonal antibodies against rat P450 1A1/2 or P450 2B1/2.
mRNA was extracted and revesely transcribed using a commercially available kit. The rat 2B1-specific primers were synthesized and the RNA concentration determined.
Test substance: alpha-Ionone, purity 90%
beta-Ionone, purity 95%
Conclusion: According to the authors, the results of the study suggest that alpha- and beta-Ionone might be potent P450 B1 inducers in rats and that both isomers may be useful for examining the role of metabolic activation in chemical-induced toxicity where metabolic activation is required.
Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, well documented and acceptable for assessment

17-MAY-2004 (158)

Type: Biochemical or cellular interactions

Remark: The study was undertaken to investigate the inhibitory effects of beta-Ionone, (-)-menthol, 1,8-cineole and alpha-terpineol on liver microsomal enzymes involved in the biotransformation of xenobiotic substances.

Result: Beta-Ionone was compared to the other test substances the most potent inhibitor of PROD (IC50 was 0.03 µM) using the Lineweaver-Burk double reciprocal plot the inhibition seemed to have been from the non-competitive type. The Ki value calculated for beta-Ionone was 89.9 nM.
EROD activities were decreased by beta-Ionone at concentrations equal or higher than 25 µM with an IC50 > 100 µM.
Beta-Ionone was only a weak inhibitor of MROD activity (IC50 was > 200 µM).

Test condition: Animals and treatment:
Female Wistar rats, weighing 250-290 g, were used. Two rats were treated with daily intraperitoneal injections of beta-naphthoflavone (B-NF) suspended in corn oil (80 mg/kg bw per day) during 4 consecutive days. This treatment schedule was regarded as sufficient to produce a marked induction of CYP1A monooxygenases. A second group of four rats was treated with phenobarbital (PB) sodium (0.1% w/v in drinking water for 4 days plus an i.p. injection of 40 mg/kg bw in the 5th day). After the last dose of B-NF or PB the animals were starved for 16 hours and sacrificed. Immediately after sacrifice, livers were quickly excised, immersed in sucrose solution and microsomal fraction prepared.
Measurements of enzyme activities:
Pentoxyresorufin-O-dependentylase (PROD), ethoxyresorufin-O-deethylase (EROD), and methoxyresorufin-O-demethylase (MROD) activities were determined. Inhibition of EROD and MROD activities were investigated in microsomes obtained from rats treated with B-NF, while inhibitory effects on PROD activity were studied in a pool of PB-induced microsomes. Test substances (beta-Ionone, cineol, menthol and terpineol) were dissolved in dimethylsulfoxide (DMSO). Inhibitors, microsomes and substrates were added to the incubation mixture (2 ml) 2 minutes prior to the start of the reaction (addition of NADPH regenerating system). The substrate concentration was kept constant at 5 µM. The concentration of the inhibitor that produces a 50% decrease (IC50) in the alkoxyresorufin-O-dealkylation activity was determined from plots of reciprocal of percentage activities vs. concentrations of the inhibitor.

Test substance: beta-Ionone, indication of purity is missing

Conclusion: The potent inhibitory effects on CYP2B1 suggest that beta-Ionone may interfere with the metabolism of xenobiotics which are substrates for this isoenzyme.

Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, well documented and acceptable for assessment

18-MAY-2004 (159)

Type: Biochemical or cellular interactions

Remark: The study was undertaken as preliminary experiment to the in chapter 5.8.2 described developmental toxicity study by the same authors to find out whether beta-Ionone given concomitantly to pentobarbital would prolong sleep. It is known that duration of pentobarbital sleep depends on the clearance of this barbiturate which is in the rat mainly due to CYP2B1 activity in the liver.

Result: Prior administration of beta-Ionone caused a dose-dependent prolongation of pentobarbital sleeping time in male and female rats. No rats of the control group treated with beta-Ionone alone slept.

Test condition: Male and non-pregnant female Wistar rats weighing approximately 220g (range: 200-250g) were used.
The rats were treated by gavage with a single dose of beta-Ionone in corn oil (males: 0, 500, 1000 an 1500 mg/kg bw; females: 0, 125, 250 and 500 mg/kg bw).
45 minutes after being orally treated with beta-Ionone the animals received an i.p. injection of pentobarbital sodium (males 35 mg/kg bw and females 25 mg/kg bw). An additional control group received only beta-Ionone. The duration of pentobarbital-induced sleep as evaluated as the time elapsed between loss and regaining of the righting reflex.

Test substance: beta-Ionone, purity 95%

Conclusion: The study indicated that concomitant administration of beta-Ionone potentiated pentobarbital induced sleeping time. According to the authors, this effect could have been due to an inhibition of CYP2B1 catalysed reactions.

Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, well documented and acceptable for assessment

18-MAY-2004 (141)

Type: Biochemical or cellular interactions

Remark: The growth of murine B16 melanomas in vitro and in vivo was investigated after treatment with certain isoprenoids (including beta-Ionone).

Result: In the in vitro experiments, beta-Ionone had IC50 values in the range of 120-150 µmol/l.
In the feeding experiments, each treatment increased ($P < 0.03$) the duration of host survival. An increased survival rate was seen with the mixture of beta-Ionone with d-gamma-tocotrienol but it did not exceed the increase seen with either material fed alone.

Test condition: The study estimated the concentrations of structurally diverse isoprenoids required to inhibit the increase in a population of murine B16(F10) melanoma cells during a 48-h incubation by 50% (IC50 value).
In the first of diet studies, experimental diets were fed to weanling female mice for 10 d prior to and 28 d following the implantation of the aggressively growing and highly metastatic B16(F10) melanoma. Melanomas were established before mice were fed experimental diets formulated with 2 mmol/kg beta-Ionone and d-gamma tocotrienol individually and in combination.

Test substance: beta-Ionone, purity 96%

Conclusion: The authors' finding that the effects of individual isoprenoids were additive suggests the possibility that one component of the anticarcinogenic action of plant-based diets

is the tumor growth-suppressive action of the diverse isoprenoid constituents of fruits, vegetables and cereal grains.

Reliability: (3) invalid
Significant methodological deficiencies

09-AUG-2004 (160)

Type: Biochemical or cellular interactions

Remark: The growth suppressive actions of beta-Ionone and gamma-tocotrienol (a mixed isoprenoid) were investigated with regard to the initiation of apoptosis and G1-phase arrest. Beta-Ionone suppressed the growth of murine B16(F10) melanoma cells (IC50 140 µM) and with greater potency, the growth of MCF-7 human breast adenocarcinoma cells (IC50 60 µM), human leukemic HL-60 cells (IC50 35 µM) and human colon adenocarcinoma (Caco-2) cells (IC5 55 µM).
Results obtained with different cell lines differing in ras and p53 status showed that the isoprenoid-mediated suppression of growth is independent of mutated ras and p53 functions. Beta-Ionone suppressed the growth of human colon fibroblasts (CCD-18Co) but only when present at 3-fold the concentration required to suppress the growth of Caco-2 cells (IC50 160 µM).

The isoprenoids initiated apoptosis and concomitantly arrested cells in the G1-phase of the cell cycle. Beta-Ionone interfered with the posttranslational processing of lamin B, an activity essential to assembly of daughter nuclei.

The conclusion of the authors was that the additive and potentially synergistic actions of the tested isoprenoids (beta-Ionone and gamma-tocotrienol) in the suppression of tumor cell proliferation and initiation of apoptosis coupled with the mass action of isoprenoid constituents of plant products might explain in part the impact of vegetable and grain consumption on cancer risk.

Test substance: beta-Ionone, purity 96%

Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, well documented and acceptable for assessment

02-JUN-2004 (161)

Type: Biochemical or cellular interactions

Remark: The objective of the study was to analyze CYP2A3 mRNA expression in different rat tissues (liver, esophagus, kidney, lung, nasal epithelium, intestine) by RT-PCR (real time polymerase chain reaction), and to study the influence of 3-methylcholanthrene, pyrazole and beta-Ionone treatment on its expression.

Result: CYP2A3 mRNA was constitutively expressed in the esophagus, lung and nasal epithelium, but not along the intestine, liver, or kidney. CYP2A3 mRNA levels were increased in the esophagus by treatment with 3-methylcholanthrene and pyrazole (17- and 7-fold, respectively), in lung by pyrazole and beta-Ionone (3- and 4-fold, respectively, although not statistically significant), in the distal part of the intestine and kidney by 3-methylcholanthrene and pyrazole, and in the proximal part

of the intestine by pyrazole. CYP2A3 mRNA was not induced in nasal epithelium, liver or in the middle part of the intestine.

Test condition: Male Wistar rats were divided into four groups of 5 rats each, and were treated i.p. for 4 days with 3-methylcholanthrene (25 mg/kg bw), pyrazole (150 mg/kg bw), beta-Ionone (1000 mg/kg bw), or vehicle.
Total RNA was extracted from tissues and CYP2A3 mRNA levels were analyzed by semiquantitative RT-PCR.

Test substance: beta-Ionone, indication of purity is missing

Conclusion: Beta-Ionone was capable of inducing CYP2A3 mRNA in the esophagus and in the lung but not in the other issues studied. The results of the study show that, in the rat, CYP2A3 is constitutively expressed in several extrahepatic tissues and its regulation occurs through a complex mechanism that is essentially tissue specific.

Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, well documented and acceptable for assessment

02-JUN-2004 (162)

Type: Biochemical or cellular interactions

Remark: In the study, the hypothesis that beta-Carotene cleavage products (CCPs) may increase oxidative stress by impairing mitochondrial function was tested in vitro.
It was found that CCPs strongly inhibit state 3 respiration of isolated rat liver mitochondria even at concentrations between 0.5 and 20 µM. This was true for retinal, beta-Ionone, and mixtures of cleavage products, which were generated in the presence of hypochlorite to mimic their formation in inflammatory regions. The inhibition of mitochondrial respiration was accompanied by a reduction in protein sulfhydryl content, decreasing glutathione levels and redox state, and elevated accumulation of malondialdehyde. Changes in mitochondrial membrane potential favor functional deterioration of the adenine nucleotide translocator.
According to the authors, these findings might reflect a basic mechanism of increasing the risk of cancer induced by CCPs.

Test substance: beta-Ionone, indication of purity is missing

Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, well documented and acceptable for assessment

18-MAY-2004 (163)

Type: Biochemical or cellular interactions

Remark: To investigate the role of metabolism in cocaine-induced immunosuppression, diazinon and beta-Ionone were administered as an esterase inhibitor and a cytochrome P450 (P450) inducer, respectively, to B6C3F1 female mice.

Beta-Ionone was administered subcutaneously for 7 consecutive days and the P450 activities were determined 3 days after the last administration. Beta-Ionone induced cocaine N-demethylation, which is the first step in the activation of cocaine to the metabolites capable of producing hepatotoxicity, as well as P450IA1- and P450IIB1-specific

monooxygenases. The inductive effects of beta-Ionone on P450IA1/2 and P450IIB1/2 proteins were confirmed by using Western immunoblotting with selective monoclonal antibodies. In addition, when beta-Ionone (600 mg/kg bw) was administered with cocaine for 7 days, the suppression of the antibody response was potentiated greatly, thymus weight was decreased significantly and serum glutamate-pyruvate transaminase was elevated.

According to the authors, these results suggest that inhibition of the esterase pathway of cocaine shunts the metabolism of cocaine into an immunotoxic pathway, and that the metabolism of cocaine by P450 may be the critical pathway for the generation of the metabolites capable of suppressing the antibody response.

Test substance: beta-Ionone, no further data on purity

Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, well documented and acceptable for assessment

02-JUN-2004 (164)

Type: Biochemical or cellular interactions

Remark: Effect of tobacco smoke compounds on the plasma membrane of cultured human lung fibroblasts.

Result: For beta-Ionone, the release of the nucleotide marker was reported to be 44%.
(For comparison: the membrane damage was classified as moderate when the nucleotide release was > 45 < 70%)

Test condition: The ability of the test material to increase the permeability of the membranes of human lung fibroblasts was studied by measuring the release of an intracellular nucleotide marker. Human diploid embryonic lung fibroblasts (line MRC-5) were cultivated to a cell density of 10 to the fifth cells/cm2. The cells were then labeled with [3H]uridine. The labelled cultures were incubated with 25 mM of the test material for 30 minutes at 37°C. Vehicle was Tris-buffered saline. A maximal release of the radioactivity was obtained by treating control cells for 30 minutes with 0.06 M sodium borate buffer and scraping with a rubber policeman. This treatment ruptured the cell membranes leaving the nuclei intact.

Test substance: beta-Ionone, indication of purity is missing

Reliability: (3) invalid
Significant methodological deficiencies

09-NOV-2004 (165)

Type: Biochemical or cellular interactions

Remark: Effects of tobacco and tobacco smoke constituents on cell multiplication in vitro.

Result: Results for beta-Ionone:
0.1 mM: 20% inhibition
1 mM: 100% inhibition

Test condition: Stem cell cultures, strain Ascites sarcoma BP8, originating from inoculated C3H mice were used to determine the toxicity of tobacco and tobacco smoke constituents. Test material was dissolved in 10 µl of ethanol and added to the test

suspension. Tests were run in duplicate. All compounds were incubated at 37°C for 48 hours. 10 µl of solvents were added to the controls. The growth rate of an incubated cell culture was calculated and compared to the average value of 8-10 controls performed in each series. The doubling time for control cultures was approximately 24 hrs. No systematic distinction was made between viable and total cell count. The effect of the tested compound was given as the ratio between the growth rates of the incubated cell culture and the controls, expressed as a percentage.

Test substance: beta-Ionone, no further data on purity

Reliability: (3) invalid
Significant methodological deficiencies

09-AUG-2004 (166)

Type: Biochemical or cellular interactions

Remark: The tumor promoting agent, 12-0-tetradecanoylphorbol-13-acetate (TPA) was found to be a both highly active stimulator of phospholipid metabolism and a potent comitogen in bovine lymphocytes. In previous studies retinoic acid and the short chain analog, beta-Ionone, antagonized TPA action, but exhibited broad dose-response curves suggesting a requirement for metabolic activation of these inhibitors.
In the present study 5,6-epoxy-beta-Ionone has been synthesized as a model metabolite and shown to be a more effective inhibitor of TPA action in vitro than the parent compound, beta-Ionone. The data are in accord with the concept that activation of both beta-Ionone and retinoic acid may proceed by epoxidation.

Test substance: 5,6-epoxy-beta-Ionone

Reliability: (3) invalid
Significant methodological deficiencies

02-JUN-2004 (167)

Type: Biochemical or cellular interactions

Remark: The effects of terpenoids (including beta-Ionone) on the activities of hepatic drug metabolizing enzymes were studied.

Result: Increases in the activities of glucuronyl transferase, biphenyl 4-hydroxylase and 4-nitrobenzoate reductase were observed. Activities of cytochrome P-450 were invreased by 50-75%. Hexobarbitone sleeping times were decreaesd to half the normal. Increased activities were maximal after 1 dose with no further increase when treatment was continued daily for 28 days. Liver weights and liver protein did not increase significantly.

Test condition: Rats were pretreated for 3 days with test material administered by intraperitoneal injection or by admixture with their food. Biphenyl 4-hydroxylase, glucuronyl transferase and 4-nitrobenzoate activities and cytochrome P-450 were determined in 10000g supernatants of the liver homogenates. Data from abstract only. Doses and vehicles not given.

Test substance: beta-Ionone, no further data on purity

Reliability: (3) invalid
Significant methodological deficiencies

17-AUG-2004 (168)

Type: Cytotoxicity

Remark: The ciliotoxicity of 316 individual compounds representative of the gaseous and semivolatile phases of tobacco smoke were investigated using chicken tracheal organ cultures.

Result: Results are time (min) to ciliostasis at 5 mM concentration for all test compounds.
The time leading to ciliostasis at a 5mM concentration was 22 min for beta-Ionone.

Test condition: Ciliotoxicity was investigated using embryo chicken tracheal organ cultures. The organ cultures were prepared aseptically from 16 day to 17 day old chicken embryos. After dissection, the trachea was rinsed, then flushed with medium and subsequently cut transversely to give rings approximately 1 mm thick. The trachea rings were transferred to petri dishes containing minimum essential medium wtih Hank's salts, Hepes and L-glutamine. One tracheal ring was then placed in a perspex testing chamber (volume 3.1 ml) that contained medium and was maintained at 37°C. A solution of test material in dimethyl sulfoxide or ethanol was then added to the chamber and time to ciliostatis was determined. Ciliary activity was studied over a 60 minute period by means of inverted microscopy using a magnification of 250.

Test substance: beta-Ionone, no further data on purity

Reliability: (3) invalid
Unsuitable test system

02-JUN-2004 (169)

Type: Cytotoxicity

Remark: The effect on cell metabolism of 320 individual smoke components were investigated by measuring their inhibition of noradrenaline induced respiration in isolated hamster brown fat cells.

Result: Materials which stimulate the basic rate of oxygen consumption, decrease the maximal noradrenaline inducible respiratory rate.

Beta-Ionone reduced noradrenaline induced respiration by 71% at a concentration of 1mM.

Test condition: The inhibition of noradrenaline induced respiration in isolated hamster brown fat cells was measured as an indication of effect on cell metabolism. The oxygen consumption rates of the cells were measured at 37°C using a Clark-type oxygen electrode fitted in a Perspex vessel of 1-ml volume. Test material was dissolved in ethanol or dimethyl sulfoxide and incubated with the cells for exactly 5 minutes during which period the oxygen consumption was registered. After this preincubation, noradrenaline was added and the oxygen consumption of the cells was registered for another 5 minutes. The noradrenaline concentration was 1 µM which is approximately twice the dose known to induce maximal respiratory rate.

Test substance: beta-Ionone, no further data on purity

Reliability: (3) invalid
Significant methodological deficiencies

02-JUN-2004 (170)

Type: Metabolism

Remark: Metabolites were isolated after gavage application from the urinary extract and identified. These products were determined for 96 hrs after the administration of beta-Ionone.

Result: The following compound were identified:
unchanged beta-Ionone, 3-oxo-beta-Ionone, 3-oxo-beta-Ionol, dihydro-3-oxo-beta-Ionol, 3-hydroxy-beta-Ionol and the glucuronides of 3-oxo-beta-Ionol and dihydro-3-oxo-beta-Ionol.

Excretion products were isolated as 2,4-dinitrophenylhydrazone derivatives (beta-Ionone, 3-oxo-beta-Ionone, 3-oxo-beta-ionol and dihydro-3-oxo-beta-Ionol) and as p-nitrobenzoate derivatives (3-oxo-beta-Ionol, dihydro-3-oxo-beta-Ionol and 3-hydroxy-beta-Ionol), which were characterized and identified by comparison with the synthetic authentic compounds. The glucuronides of 3-oxo-beta-Ionol and dihydro-3-oxo-beta-Ionol were detected in the urine. The latter compound was isolated as free glucuronide, sodium salt and 2,4-dinitrophenylhydrazone.

Test condition: ADMINISTRATION
The test material, in aqueous suspension with gum arabic, was given by stomach tube to a male albino rabbit (approximately 3.0 g/kg bw; approximate total dose of 23 g) daily for 7 days. Urine was collected daily during dosing and for 4 days after last dose.

EXTRACTION
The urine was adjusted to pH 6.0 and extracted with ether. Free metabolites and their derivatives were separated by TLC and identified by UV and IR spectra.

Test substance: beta-Ionone; no further data on purity provided

Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, well documented and acceptable for assessment.

09-AUG-2004 (171)

Type: Metabolism

Remark: The urinary metabolites of beta-Ionone after oral administration to rabbits were studied.

Result: After feeding the rabbits with beta-Ionone 4-oxo-beta-Ionone and 4-oxo-beta-Ionole were identified as metabolites in the urine of the animals.

Test condition: 100 g beta-Ionone was fed to 2 rabbits for a period of 18 days (daily dose about 2.8 g/animal). The urine was collected. The metabolites were extracted from the urine, separated by chromatography and identified by chemical reaction with phenyl semicarbazide and 2,4-dinitrophenyl hydrazine.

Test substance: beta-Ionone, no further data on purity

Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, well documented and acceptable for assessment

09-AUG-2004 (112)

Type: Toxicokinetics

Remark: As part of a corresponding study on the effects of test material on the motility of mice, concentrations of test material in blood samples were determined after inhalation exposure.

Result: A trace (less than 0.1 ng/ml) concentration of beta-Ionone was detected in blood samples. No further details provided.

Test condition: Groups of four female outbred Swiss mice with mean body weights of 28.5 grams were used. Both 4-6 week old and 6 month old mice were used. Air was passed into the cage through a glass tube containing 1.5 ml test material. Total test material volume was 20-50 mg. Blood samples were taken from the animals after 0, 30, 60 and 90 minutes of inhalation exposure. Plasma was extracted and the sera were investigated by gas chromatographic-spectroscopic systems to identify and quantify the test materials. Results provided are after 1 hour of exposure.

Test substance: beta-Ionone, no further data on purity

Reliability: (3) invalid
Significant methodological deficiencies

09-AUG-2004 (148)

Type: other: chemoprevention

Remark: Beta-Ionone at a dose level of 1000 ppm was examined for protection against aflatoxin B1 (AFB1) hepatocarcinogenesis, induction of the mixed function oxidase (MFO) system and metabolism of AFB1 in rainbow trout.

Result: Average body weight and avarage liver weight in the beta-Ionone treated fish was not significantly different from controls. Beta-Ionone was not effective at reducing the tumor incidence (35% versus 38% in AFB1 exposed controls).

Test condition: The compound was fed to fingerling rainbow trout for 8 weeks. At that time the activity of the MFO system and cytochrome P450 content were measured and the trout were exposed for 2 weeks to 20 ppb AFB1 in the same diets. After feeding the test diets without AFB1 for another 6 weeks and basal diet for another 52 weeks, the tumor incidence was determined.

Test substance: beta-Ionone, no further data on purity

Reliability: (3) invalid
Unsuitable test system

09-AUG-2004 (172)

Type: other: chemoprevention

Remark: Efficacy of beta-Ionon in the chemoprevention of rat mammary carcinogenesis was examined.

Result: WEIGHT GAIN
Was not influenced by the treatment

TUMOR INCIDENCE, LATENCY AND MULTIPLICITY
During the 151 days following the administration of DMBA, the presence of a tumor was confirmed in 91% and 41%, respectively of the rats in the control and beta-Ionone groups. The substance substantially increased tumor latency (> 151 days versus 74 days in controls). The tumor multiplicity was significantly decreased (0.7 tumors/rat versus 2.9 tumors/rat

in controls).

HISTOPATHOLOGY
At the termination of the study, 85% (17/20) and 24% (6/25) respectively, in the control and beta-Ionone groups had palpable tumors. One histologically confirmed tumor was present in each of 17 control rats. The tumors consisted of 63% adenocarcinoma, 16% adenoma, 10% fibroadenoma and 10% benign masses. The 9 tumors tumors excised from 6 beta-Ionone treated rats consisted of 4 (44%) adenocarcinoma, 2 (22%) adenoma and 3 (33%) benign masses.

Test condition: Female Sprague-Dawley rats, 30-35 days of age were fed after a acclimitization period an experimental diet containing 36 mmol/kg beta-Ionone (dose calculation: 192 mg/mmol (molecular weight) x 36 mmol/kg = ca. 6900 ppm; ca. 345 mg/kg bw).
The rats were fed this diet for 2 weeks. Tumors were then induced as described by Huggins (Exp. Leukemia and Mammary Cancer, Univ. Chicago Press, Chicago, IL, 1979) with the exception that the level of Dimethylbenzanthracene (DMBA) was reduced by 50% to increase tumor latency. Specifically the rats at 54 days of age were given a single gastric intubation of a suspension of DMBA in sesame oil (65 mg/kg bw). The dietary regimens were continued for 22 weeks with the diet being replaced at 2-day intervals. The rats were weighed at 6-10 day- intervals and starting on day 44 post-DMBA the rats were palpated for the presence of soft tissue masses (tumors) at 3-5 day intervals.

Test substance: beta-Ionone, purity 95%

Conclusion: According to the authors, beta-Ionone was a highly effective non-toxic anticarcinogenic agent under the conditions of the study.

Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, well documented and acceptable for assessment.

25-APR-2005 (173) (151)

Type: other: effect on olfactory tissue

Remark: Rats were exposed to a variety of odorous compounds from 2 weeks of age for periods from 1-12 weeks and changes olfactory bulb were investigated. Beta-Ionone was one of the 44 test substances.

Result: Moderate changes noted. Lateral and medial areas affected. [Exposure concentration was 1.6 x 10(-9) M]. No further details on results given.

Test condition: Wistar rats were raised in cylindrical lucite cages with closed tops. Fresh air was blown through filters of charcoal and molecular sieves. The flow of air was monitored with a venturimeter for each of two sets of 6 cages and kept at about 0.6 l/sec through each cage. Each substance was introduced into the air stream from a glass bottle, the content of which was weighed before and after the experiments to measure the concentration of substance in the stream.
The rats weighed between 28 and 39g and were about 2 weeks old. For every 5 substances there was a group of control animals exposed to filtered fresh air only.
The animals were sacrificed at about 1, 2, and 3 months of age. For each odour at least two different exposure times were used (beta-Ionone: 1 and 5 weks). 4 animals were used per

group. The animals were then perfused in buffered saline and fixed with a formaldehyde-glutaraldehyde mixture. The emphasis was placed upon the distribution of changes in the mitral cells of the olfactory bulbs following exposure to different odours. The morphological changes noted were principally a darkening and shrinkage of the cell bodies (both cytoplasm and nucleus) as observed by light microscope.

Test substance: beta-Ionone, no further data on purity

Reliability: (3) invalid
Significant methodological deficiencies

09-AUG-2004 (174)

Type: other: in vitro teratogenicity

Remark: Review article on the in vitro teratogenicity screening using the "rat embryo limb cells in culture" assay.
Beta-Ionone had no effect on cell differentiation (number of differentiated chondrogenic foci per micromass island) and cytolethality (neutral red spectrophotometric estimation of surviving cells). Results only in tabular format without further details (test concentrations etc).

Test substance: beta-Ionone, no further data on purity

Reliability: (4) not assignable
Secondary literature

09-AUG-2004 (175)

Type: other: review

Remark: beta-Ionone was reviewed at the 11th meeting of the Joint FAO/WHO Expert Committee on Food Additives, specifications were prepared and a conditional acceptable daily intake for man (ADI) of 0-0.1 mg/kg bw/day was established.

Reliability: (2) valid with restrictions
Data from Handbook or collection of data

02-JUN-2004 (176)

Type: other: review

Remark: beta-Ionone was reviewed by the Joint FAO/WHO Expert Committee on Food Additives, specifications were prepared and a conditional acceptable daily intake for man (ADI) of 0-0.1 mg/kg bw/day was established.

Reliability: (2) valid with restrictions
Data from Handbook or collection of data

02-JUN-2004 (177)

Type: other: review

Remark: Review on description, physical properties, occurrence, status and biological data.
Both alpha- and beta-Ionone were granted GRAS status by FEMA (1965) and are approved by the FDA for food use (21 CFR 121.1164). The council of Europe (1974) listed alpha and beta-Ionone giving ADIs of 0.1 mg/kg bw/day for both. The Joint FAO/WHO Expert Committee on Food additives has published monographs and specifications for both isomers, giving conditional ADIs of 0-0.1 mg/kg bw/day.

Typical use concentrations in final products are 0.03% (soap), 0.003% (detergent), 0.016% (creams, lotions) and 0.3% (perfume).

Reliability: (2) valid with restrictions
Data from Handbook or collection of data

16-NOV-2004 (178)

Type: other: review

Remark: A review was conducted to determine the margins of safety between no-observed-effect levels (NOELs) and daily per capita intake of flavouring substances evaluated by the Joint FAO/ WHO Expert Committee on Food Additives (JECFA) using the safety evaluation procedure for flavouring substances.
The intake of beta-Ionone was estimated to be 2.53 x 10-3 mg/kg bw/day (highest intake estimate selected from European or US per capita intake data).
Using a NOEL of 10 mg/kg bw/day from a repeated dose toxicity study (Ford et al., 1983) a margin of safety of 3947 can be calculated.

Test substance: beta-Ionone

Reliability: (2) valid with restrictions
Data from Handbook or collection of data

15-JUN-2004 (179)

Type: other: review

Remark: The Joint FAO/WHO Expert Committee (JECFA) evaluated a group of 21 flavouring agents that includes alpha- and beta-Ionone and structurally related substances.
Estimated intake values for Ionones and related substances were reported in tabular format. Per capita intake was estimated from data derived from surveys in Europe (International Organization of the Flavor Industry, 1994) and the United States (US Academy of Sciences, 1989).
For beta-Ionone the estimated daily per capita intake was 150 µg in Europe and 100 µg in USA. The substance was classified in structural class I according to Cramer et al. (1978). For class I substances threshold of [toxicological] concern is 1800 µg/day.
The ADI values previously established for alpha- and beta-Ionone were maintained at the meeting (0-0.1 mg/kg bw).
Based on exposure data and available toxicological information it was concluded that there are no safety concerns for beta-Ionone (used as a flavouring substance).

Test substance: Beta-Ionone

Reliability: (2) valid with restrictions
Data from Handbook or collection of data

02-JUN-2004 (180)

6.1 Methods Handling and Storing

Safe Handling: Protection against fire and explosion:
The relevant fire protection measures should be noted.

Storage Req.: Keep container tightly closed in a cool, well-ventilated place.

Transport Code: RM

Remark: Personal protective equipment

Respiratory protection:
Breathing protection if gases/vapours are formed. Particle filter Type P2 or FFP2, (medium efficiency for solid and liquid particles e.g. EN143, 149).

Hand protection:
Chemical resistant protective gloves (EN 374)
Suitable materials also with prolonged, direct contact (Recommended: Protective index 6, corresponding > 480 minutes of permeation time according to EN 374):
nitrile rubber (NBR) - 0.4 mm coating thickness
Supplementary note: The specifications are based on own tests, literature data and information of glove manufacturers or are derived from similar substances by analogy. Due to many conditions (e.g. temperature) it must be considered, that the practical usage of a chemical-protective glove in practice may be much shorter than the permeation time determined in accordance with EN 374.
Manufacturer's directions for use should be observed because of great diversity of types.

Eye protection:
Safety glasses with side-shields (frame goggles) (EN 166)

Body protection:
Body protection must be chosen depending on activity and possible exposure, e.g. apron, protecting boots, chemical-protection suit (according to DIN-EN 465).

General safety and hygiene measures:
Handle in accordance with good industrial hygiene and safety practice.

TRANSPORT INFORMATION

Land transport
ADR :Class 9
Packaging group III
UN-number 3082
Designation of goods ENVIRONMENTALLY HAZARDOUS SUBSTANCE, LIQUID, N.O.S. (Contains: BETA-JONON)
RID : Class 9
Packaging group III
UN-number 3082
Designation of goods ENVIRONMENTALLY HAZARDOUS SUBSTANCE, LIQUID, N.O.S. (Contains: BETA-JONON)

Inland waterway transport

ADNR : Class 9
Packaging group III
UN-number 3082
Designation of goods ENVIRONMENTALLY HAZARDOUS SUBSTANCE, LIQUID, N.O.S. (Contains: BETA-JONON)

Sea transport
IMDG/GGVSee : Class 9
Packaging group III
UN-number 3082
Marine pollutant YES
Exact technical name ENVIRONMENTALLY HAZARDOUS SUBSTANCE, LIQUID, N.O.S. (contains BETA-JONON)

Air transport
ICAO/IATA : Class 9
Packaging group III
UN-number 3082
Exact technical name ENVIRONMENTALLY HAZARDOUS SUBSTANCE, LIQUID, N.O.S. (contains BETA-JONON)

Flag: non confidential, Critical study for SIDS endpoint
10-NOV-2004 (13)

Add. Information: Best before: In sealed original containers under nitrogen 24 months.

Test substance: beta-Ionon R (BASF)
Flag: non confidential, Critical study for SIDS endpoint
18-MAR-2004 (2)

6.2 Fire Guidance

Prot. Equipment: Wear a self-contained breathing apparatus.
Ext. Medium: dry extinguishing media, carbon dioxide, foam
Add. Information: Dispose of fire debris and contaminated extinguishing water in accordance with official regulations.

Flag: non confidential, Critical study for SIDS endpoint
17-AUG-2004 (13)

6.3 Emergency Measures

Type: other: General advice: Remove contaminated clothing.

Flag: non confidential, Critical study for SIDS endpoint
09-DEC-2003 (13)

Type: injury to persons (inhalation)

Flag: non confidential, Critical study for SIDS endpoint
09-DEC-2003 (13)

Type: injury to persons (skin)

Flag: non confidential, Critical study for SIDS endpoint
09-DEC-2003 (13)

Type: injury to persons (eye)

Flag: non confidential, Critical study for SIDS endpoint
09-DEC-2003 (13)

Type: injury to persons (oral)

Flag: non confidential, Critical study for SIDS endpoint
09-DEC-2003 (13)

Type: accidental spillage

Remark: Personal precautions:
Ensure adequate ventilation.

Environmental precautions
Do not empty into drains.

Methods for cleaning up or taking up:
For small amounts: Pick up with suitable absorbent material (e.g. sand, sawdust, general-purpose binder, kieselguhr).
Dispose of absorbed material in accordance with regulations.
For large amounts: Pump off product.

Flag: non confidential, Critical study for SIDS endpoint
17-AUG-2004 (13)

Type: other: Note to physician

Remark: Treatment:
Symptomatic treatment (decontamination, vital functions).

Flag: non confidential, Critical study for SIDS endpoint
10-NOV-2004 (13)

6.4 Possib. of Rendering Subst. Harmless

6.5 Waste Management

Memo: other: Observe national and local legal requirements.

Flag: non confidential, Critical study for SIDS endpoint
10-NOV-2004 (13)

6.6 Side-effects Detection

6.7 Substance Registered as Dangerous for Ground Water

6.8 Reactivity Towards Container Material

(1) BASF AG, Product Database Fine Chemicals, status, 18 March 2004

(2) BASF AG, Technical Data Sheet beta-Ionon R, ME 018 d/e, 08/2003

(3) BASF AG, General Info BETA-IONONE R, status 18 March 2004

(4) ROEMPP Online, © 2003 Georg Thieme Verlag, status 18 March 2004

(5) Ullmann's Encyclopedia of Industrial Chemistry, Sixth Edition, 2000 Electronic Release, 2000 Wiley-VCH Verlag GmbH, Weinheim, Germany

(6) Handbook of Environmental Data on Organic Chemicals, 4th Edition, Copyright ©2001 by John Wiley & Sons, Inc.

(7) BASF AG, Analytical report, unpublished data, H-NMR spectra of beta-Ionone (BASF AG R Ch81721616K0), 26 Aug. 2004

(8) BASF AG, Analytical report, unpublished data, H-NMR spectra of beta-Ionone (Givaudan, E 500/99), 26 Aug. 2004

(9) BASF AG, Analytical report, unpublished data, H-NMR spectra of beta-Ionone (beta-Ionen DSM), 26 Aug. 2004

(10) Knovel Critical Tables Copyright: © 2003

(11) Personal internal communication, 07 May 2004

(12) BASF AG, 2004, internal communication.

(13) BASF AG, Safety data sheet BETA-IONON R, 27 Aug. 2004

(14) The Dictionary of Substances and Their Effects (DOSE, 2nd Electronic Edition)2004 by The Royal Society of Chemistry/Knovel Corp.

(15) Ariel WebInsight® Chemical Database, status 09 Dec 2003

(16) List of MAK- and BAT-values 2003 (Notification 39), 01 July 2003, WILEY-VCH Verlag GmbH, Weinheim, Germany

(17) Catalogue of Substances Hazardous to Water - Umweltbundesamt Berlin, status 24 Sept 2003

(18) Statutory order on hazardous incidents (Germany), 26 Apr. 2000

(19) National Chemical Inventories, 2003 Issue 1

(20) BASF AG, 2004, internal communication with Department GUU/LE (data from German Emission Register 2000)

(21) Beilstein, Handbook (online), BRN 1909545, BPR 79-77-6, Pfizer & Co. US 2638483, 1951, Handbook

(22) BASF AG, 2005, Product Safety, data assessment, unpublished

calculation, SRC MPBPWIN 1.41, 15 Aug. 2005

(23) Baglay A.K., Gurarly L.L. and Kuleshov G.G., 1988, Physical properties of compounds used in vitamin synthesis, J. Chem. Eng. Data, 33, 512-518

(24) Serpinsky V.V., Voitkevich S.A. and Luboshic N., 1957, Yu. Zh. Fiz. Khim. 31, 1278. Cited by Baglay A.K., Gurariy L.L. and Kuleshov G.G., 1988, Physical properties of compounds used in vitamin synthesis, J. Chem. Eng. Data, 33, 512-518

(25) Fichan I., Larroche C. and Gros J.B., 1999, Water solubility, vapor pressure, and activity coefficients of terpenes and terpenoids, J. Chem. Eng. Data 44, 56-62

(26) BASF AG, 1989, Analytical Laboratory, Dampfdruck 4-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-3-buten-2-on), unpublished study, report No. BRU 89.178, 06 July 1989

(27) BASF AG, 1982, Analytical Laboratory, Dampfdruck von beta-Ionon, unpublished study, J.Nr. 47-1028, 27 Aug. 1982

(28) Baglay A.K., Gurariy L.L. and Kuleshov G.G., 1988, Physical properties of compounds used in vitamin synthesis, J. Chem. Eng. Data, 33, 512-518

(29) BASF AG, 1989, Analytical Laboratory, Bestimmung des Verteilungskoeffizienten Log Pow der Prüfsubstanz in 1-Octanol/Wasser bei 25°C, unpublished study, J.Nr. 106288/11, 02 May 1989

(30) Givaudan, 1994, Corporate Safety & Environmental Affairs, Partition coefficient n-octanol/water of IONONE BETA SYNT according to OECD Guideline No. 117, unpublished study, 02 Nov. 1994

(31) BASF AG, 2003, Product Safety, data assessment, unpublished calculation, SRC KOWWIN 1.66, 4 Dec. 2003

(32) BASF AG, 1989, Department of Technical Development and Technology, Sicherheitstechnische Kenngrößen, unpublished study, SIK-Nr. 89/0546, 16 June 1989

(33) BASF AG, 2000, Department of Technical Safety, Absence of explosive and oxidizing properties of (E)-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-3-buten-2-one, internal communication, expert judgement, 20 Dec 2000

(34) Okahata Y. and Ebato H., 1992, Detection of bioactive compounds using a lipid-coated quartz-crystal microbalance, Trends Analyt. Chem. 11, 344-354

(35) BASF AG, 2003, Product Safety, SRC AOP v1.90, data assessment, unpublished calculation, 4 Dec. 2003

(36) BASF AG, 2003, Product Safety, SRC AOP v1.90, data assessment, unpublished calculation, 9 Dec. 2003

(37) BASF AG, Department of Product Safety, Expert Judgment on Hydrolysis, 10 Nov. 2004

(38) Guardiola J., Ventura F. and Rivera J., 1989, Occurrence of industrial organic pollution in a groundwater supply: screening, monitoring and evaluation of treatment processes, Water Supply 7, 11-16

(39) Juettner, F.,1995, Elimination of terpenoid odorous compounds by slow sand and river bank filtration of the Ruhr river in Germany. Wat. Sci. Tech. 31(11), 211-217

(40) Wilkesmann, R., Schlett, C. and H.P. Thier, 1995, Determination of ultra traces of odors in water after solid phase extraction and closed-loop stripping analysis (CLSA), Vom Wasser 84, 357-367.

(41) Rogge W.F., Hildemann L.M., Mazurek, M.A. and Cass G.C., 1993, Sources of fine organic aerosol. 4. Particulate abrasion products from leaf surfaces of urban plants, Environ. Sci. Technol. 27, 2700-2711

(42) Ferreira V., Ardanuy M., López R. and Cacho J.F., 1998, Relationship between flavor dilution values and odor unit values in hydroalcolholic solutions: role of volatility and a practical rule for its estimation. J. Food Chem. 46, 4341-4346

(43) Salt S.D., Tuzun S. and Kuc J., 1986, Effects of ß-ionone and abscisic acid on the growth of tobacco and resistance to blue mold. Micry of effects of stem infection by Peronospora tabacina Adam, Physiol. Molecul. Plant Pathol. 28, 287-297

(44) Juettner F., 1984, Dynamics of the volatile organic substances associated with cyanobacteria and algae in a eutrophic shallow lake, Appl. Environ. Microbiol. 47, 814-820

(45) Chorus I., Klein G., Fastner I. & Rotard W., 1992, Off-flavours in surface waters - how efficient is bank filtration for their abatement in drinking water? Water Sci. Technol. 25, 251-258

(46) Jones G.J. and Korth W., 1995, In situ production of volatile odour compounds by river and reservoir phytoplankton populations in Australia, Water Sci. Technol. 31, 145-151

(47) Bao M.-L., Barbieri K., Burrini D., Griffini O. and Pantani F., 1997, Determination of trace levels of taste and odor compounds in water by microextraction and gas-chromatography-ion-trap detection-mass spectrometry, Water Res. 31, 1719-1727

(48) MacTavish H., Davies N.W. and Menary R.C., 2000, Emission of volatiles from brown boronia flowers: some comparative observations, Ann. Botany 86, 347-354

(49) Hansen M., Buttery R.G., Stern D.J., Cantwell M.I. and Ling L.C., 1992, Broccoli storage under low-oxygen atmosphere: identification of higher boiling volatiles, J. Agric. Food Chem. 40, 850-852

(50) Zeringue Jr. H.J., 1997, Volatile antifungal compounds in maize kernels: effects of ear position on aflatoxin production, J. AOAC Internat. 80, 341-344

(51) MacTavish H. and Menary R.C., 2000, Production of volatiles in brown boronia flowers after harvest: pilot scale research, J. Horticult. Sci. & Biotechnol. 75, 455-458

(52) MacTavish H.S. and Menary R.C., 1997, Volatiles in different floral organs, and effect of floral characteristics on yield of extract from Boronia megastigma (Nees), Ann. Botany 80, 305-311

(53) Plummer J.A., Wann J.M. and Spadek Z.E., 1999, Intraspecific variation in oil components of Boronia megastima Nees (Rutaceae) flowers, Ann. Botany 83, 253-262

(54) Roberts N.J. and Menary R.C., 1994, Effect of nitrogen on growth, flower yield, oil composition, and yield in Boronia megastima Nees, J. Plant Nutrit. 17, 2035-2052

(55) Plummer J.A., Wann J.M., Orifici R. and Spadek Z.E., 1998, Effects of photon flux density on photosynthesis, growth, flowering, and oil content in Boronia, Aust. J. Agric. Res. 49, 791-797

(56) Lazari D.M., Skaltsa H.D. and Constantinidis T., 2000, Volatile constituents of Cerastium candidissimum, a Greek endemic species, Flav. Fragr. J. 15, 174-176

(57) Angelopoulou D., Demetzos C., Dimas C., Perdetzoglou D. and Loukis A., 2001, Essential oils and hexane extracts from leaves and fruits of Cistus monspeliensis - cytotoxic activity of ent-13-epi-manoyl oxide and its isomers, Planta Med. 67, 168-171

(58) Tellez M., Khan I.A., Kobaisy M., Schrader K.K., Dayan F.E. and Osbrink W., 2002, Composition of the essential oil of Lepidium meyenii (Walp.), Phytochemistry 61, 149-155

(59) Flamini G., Cioni P.L., Morelli I., Ceccarini L., Andolfi L. and Macchia M., 2003, Composition of the essential oil of Medicago marina L. from the coastal dunes of Tuscany, Italy, Flav. Fragr. J. 18, 460-462

(60) Ômura H., Honda K. and Hayashi N., 2000, Floral scent of Osmanthus fragrans discourages foraging behavior of cabbage butterfly, Pieris rapae, J. Chem. Ecol. 26, 655-666

(61) Anonymous, 2001, Indian roses smell different, COSSMA 3/2001, 44-45

(62) Puri H.S., Siddiqui M.S., Akhila A. and Naqvi A., 1985, Essential oil of Thymus serpyllum: a source of thymol in India, Parfümerie und Kosmetik, 66, 606-609

(63) Evans W.G., 1994, Volatile organic chemicals of a shore-dwelling cyanobacterial mat community, J. Chem. Ecol. 20, 219-230

(64) Cotsaris E., Bruchet A., Mallevialle J. and Bursill D.B., 1995, The identification of odorous metabolites produced from algal monocultures, Water Sci. Technol. 31, 251-258

(65) Moehren S. and Juettner F., 1983, Odorous compounds of different strains of Anabaena and Nostoc (Cyanobacteria), Water Sci. Technol. 15, 221-228

(66) Walsh K., Jones G.J. and Dunstan R.H., 1998, Effect of high irradiance and iron on volatile odour compounds in the cyanobacterium Microcystes aeruginosa, Phytochemistry 49, 1227-1239

(67) Hesselberg R.J. and Seelye J.G., 1982, Identification of organic compounds in Great Lake fishes by gas chromatography/mass spectrometry: 1977, Administrative Report No. 82-1, U.S. Fish and Wildlife Service, Great Lakes Fishery Laboratory

(68) Tatsuka K., Suekane S., Sakai Y. and Sumitani H., 1990, Valatile constituents of kiwi fruit flowers: simultaneous distillation and extraction versus headspace sampling, J. Agric. Food Chem. 38, 2176-2180

(69) Ricking M., Schwarzbauer J. and Franke C., 2003, Molecular markers of anthropogenic activity in sediments of the Havel and Spree Rivers (Germany), Water Res. 37, 2607-2617

(70) Peterson R.J. and Chang S.S., 1982, Identification of volatile flavor compounds of fresh, frozen beef stew and a comparison of these with those of canned beef stew. J. Food Sci. 47, 1444

(71) Peterson R.J., Izzo H.J., Jungermann E. and Chang S.S., 1975, Changes in volatile flavour compounds during the retorting of canned beef stew, J. Food Sci. 40, 948

(72) Shahidi F., Rubin L.J. and D'Souza L.A., 1986, Meat flavour volatiles: a review of the composition, techniques of analysis, and sensory evaluation, CRC Crit. Rev. Food Sci. Nutr. 24, 141-243

(73) Boyle L.L., Cullough N.G.M. and van Poppelen P., 1996, Ozonation of sludge-press liquors: determination of carbonyl compounds by the PFBOA method and the effect on the chemical oxygen demand. Div. Environ. Chem. Preprints Extend. Abstracts 36 (2), 70-74

(74) Juettner F., 1992, Flavour compounds in weakly polluted rivers as a means to differentiate pollution sources. Water Sci. Technol. 25, 155-164

(75) BASF AG, 2003, Product Safety, data assessment, unpublished calculation, SRC PCKOCWIN v1.66, 4 Dec. 2003

(76) TGD, 2003. Technical Guidance Document on Risk Assessment in support of Commission Directive 93/67/EEC on risk assessment of new notified substances, Commission Regulation (EC) No 1488/94 on risk assessment for existing substances and

Directive 98/8/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market. Part III. European Commission Joint Research Centre. EUR 20418 EN/3.

(77) Thomas R.G., 1982, Volatilization from water, pp. 15.1-15.34. In: Handbook of chemical property estimation methods, environmental behavior of organic compounds. Lyman W.J., Reel, W.F., Rosenplatt, D.H. (eds), McGraw-Hill Book Company, New York

(78) BASF AG, 2003, Product Safety, data assessment, unpublished calculation, SRC HENRYWIN v3.10, 4 Dec. 2003

(79) BASF AG, Product Safety, data assessment, unpublished calculation, Mackay Level I V2.11, 09 Nov. 2004

(80) Takasago International Cooperation, 1999, Safety Assessment Lab., Central Research Laboratory, Biodegradability on beta-ionone, 25 May 1999

(81) Roche, data compilation, ecotoxicological evaluation, beta-ionone, unpublished data, E-29/89, 02 Aug. 1989

(82) BASF AG, Laboratory of Ecology, 1989, Pruefbericht ueber eine Untersuchung auf biologische Abbaubarkeit im respirometrischen Test (EG-Methode), Test No. 388429, unpublished study, 02 Mar 1989

(83) RIFM, 2000, Research Institute on Fragrance Materials Inc., Ionone Beta: Determination of ready biodegradability (CO2-Evolution test), unpublished study, No. BL6803/B, finalised at 3rd May 2000

(84) Korth W., Ellis J. and Bowmer K., 1992, The stability of geosmin and MIB and their deuterated analogues in surface waters and organic solvents, Water Sci. Technol. 25, 115-122

(85) BASF AG, 2003, Product Safety, SRC BCFWIN V2.14, data assessment, unpublished calculation, 4 Dec. 2003.

(86) Veith G.D., Defoe D.L. and Bergstedt B.V., 1979, Measuring and estimating the bioconcentration factor of chemicals in fish. J. Fish Board Can. 36, 1040-1948

(87) Geiger D.L., Call D.J. and Brooke, L.T., 1988, Acute toxicities of organic chemicals to fathead minnows (Pimephales promelas), Vol. IV. Center for Lake Superior Environmental Studies, University of Wisconsin.

(88) Hoffmann LaRoche Inc., data compilation, ecotoxicological evaluation, beta-ionone, unpublished data, E-29/89, 02 Aug. 1989

(89) BASF AG, 1989, Department of Toxicology, Golden Orfe (Leuciscus idus L., golden variety) - Report on the study of the acute toxicity, unpublished report 10F0606/885329, 10 June 1989.

(90) BASF AG, 2004, Product Safety, beta-Ionon R - Determinaiton

of the acute effect on the swimming ability of the water flea Daphnia magna STRAUS, unpublished study, 50E0449/023002, 19 Aug 2004.

(91) BASF AG, 1990, Department of Product Safety, Laboratory of Ecology, Akute Toxizität für Daphnien nach DIN 38412 L11, unpublished study, 01/89/1485, 15 Jan 1990

(92) ToxRatPro, Version 2.08, Statistical evaluation of an acute daphnid test, unpublished calculation, 30 Jan 2004

(93) BASF AG, Department of Product Safety, Laboratory of Ecology/Toxicology, Untersuchungsbericht Prüfung auf Ökotoxizität: Hemmung der Algen-Zellvermehrung nach DIN 38412 L9, unpublished study, 01/89/1485, 6 Dec 1989

(94) Tallarida R.S. and Jacob L.S., 1979, The dose-response relation in pharmacology, Springer, pp 98-103

(95) ToxRatPro, Version 2.08, Statistical evaluation of an algal growth test, unpublished calculation, 02 Feb 2004

(96) Ikawa M., Sasner J.J. and Haney J.F., 2001, Activity of cyanobacterial and algal odor compounds found in lake waters on green alga Chlorella pyrenoidosa growth, Hydrobiologia 443, 19-22

(97) BASF AG, 1988, Laboratory of Ecology, Oxygen consumption according to Robra, unpublished study, Study No. 0909/88, 1988

(98) Givaudan, 1991, Group Safety & Environmental Protection, IONONE BETA SYNTHETIC "Activated Sludge, Respiration Inhibition Test", unpublished study, 19 Feb. 1991

(99) BASF AG, Laboratory of Ecology, 1988, Pruefbericht ueber eine Untersuchung der Wirkung von beta-Ionon auf die Atmungsaktivitaet von Belebtschlamm, No. 01.0429, unpublished study, 1988

(100) Utama I.M.S., Wills R.B.H., Ben-Yehoshua S. and Kuek C., 2002, In vitro efficacy of plant volatiles for inhibiting the growth of fruit and vegetable decay microorganisms, J. Agric. Food Chem., 50, 6371-6377

(101) Kim J., Marshall M.R. and Wei C., 1995, Antibacterial activity of some essential oil components against five foodborne pathogens, J. Agricult. Food Chem. 43, 2839-2845

(102) Kubo I., Muroi H. and Himejima M., 1992, Antimicrobial activity of green tea flavor components and their combination effects, J. Agricult. Food Chem. 40, 245-248

(103) Wei C., Tan H., Fernando S.Y. and Ko N.-J., 1986, Inhibitory effect of beta-ionone on growth and aflatoxin production of Aspergillus parasiticus on peanuts, J. Food Protection 49, 515-518

(104) Cohen Y., Pe'er S., Balass O. and Coffey M.D., 1987, A fluorescent technique for studying growth of Peronospora

tabacina on leaf surfaces, Phytopathology 77, 201-204

(105) Schafer E.W., Bowles W.A.Jr. and J. Hurlbut, 1983, The acute oral toxicity, repellency, and hazard potential of 998 chemicals to one or more species of wild and domestic birds, Arch. Environ. Contam. Toxicol. 12, 355-382

(106) Larroche C., Creuly C. and Gros J.-B., 1995, Fed-batch biotransformation of beta-ionone by Aspergillus niger, Appl. Microbiol. Biotechnol. 43, 222-227

(107) Larroche C. and Gros J.-B., 1997, Special transformation processes using fungal spores and immobilized cells, Adv. Biochem. Eng. Biotechnol. 55, 179-220

(108) Grivel F., Larroche C. and Gros J.B., 1999, Determination of the reaction yield during biotransformation of the volatile and chemically unstable compound beta-ionone by Aspergillus niger, Biotechnol. Prog. 15, 697-705

(109) Grivel F. and Larroche C., 2001, Phase transfer and biocatalyst behaviour during biotransformation of beta-ionone in a two-phase liquid system by immobilised Aspergillus niger, Biochem. Eng. J. 7, 27-34

(110) Meylan W.M., Howard P.H. and Boethling R.S., 1996, Improved method for estimating water solubility from octanol/water partition coefficient, Environ. Toxicol. Chem. 15, 100-106.

(111) Ide H. und Toki S.: Biochem. J., 119, 281-287, (1970)

(112) Prelog V and Meier HL (1950). Über die biochemische Oxydation von beta-Ionon im Tierkörper, Helv. Chim. Acta 33, 1276-1285

(113) Jenner et al. (1964). Cited in Opdyke DLJ (1979). Monographs on fragrance raw materials, Ionone, 444

(114) Jenner PM, Hagan EC, Taylor JM, Cook EL, Fitzhugh OG (1964). Food flavourings and compounds of related structure I. Acute oral toxicity. Fd. Cosmet. Toxicol. 2, 327-343

(115) Flavor and Extract Manufacturers' Association of the United States, DC (1979), Scientific literature review of alicyclic compounds of carbon, hydrogen and oxygen in flavor use - 4th supplement, prepared for US Food and Drug Administration, PB-298385, April 1979

(116) Sporn A, Schöbesch O, Marin V, Panaitescu E and Runcanu L (1963). Igiena 12, 437-446

(117) Sporn et al. (1963) cited in Opdyke DLJ (1979). Monographs on fragrance raw materials, Ionone, 444

(118) Wenzel DG and Ross CR (1957). Central Stmulating properties of some terpenones. J. Am. Pharm. Assoc. 46, 77-82

(119) BASF AG, Department of Toxicology, Report on the acute dermal irritation / corrosivity to the intact dorsal skin of beta-Ionone in white rabbits, unpublished report, project

no. 18H0245/912113, 13 May 1992

(120) Hoffmann-LaRoche Inc (1967). Acute toxicity, eye and skin irritation tests on aromatic compounds, unpublished results, 16 Nov. 1967

(121) Takasago International Corporation (1999). Beta-Ionone (CAS-no.: 14901-07-6). Toxicology studies in the Guinea pig. Final report. Acute dermal irritation / photoxicity study, skin sensitisation: Magnusson and Kligman Maximisation, unpublished report (test no. P-4908), English description, August 4, 1999

(122) BASF AG, Department of Toxicology, Report on the acute irritation to the eye of beta-Ionone in white rabbits, unpublished report, project no. 18H0245/912114, 13 May 1992

(123) Klecak G (1985). The Freund's complete adjuvant test and the Open Epicutaneous Test, Curr. Probl. Derm. 14, 152-171, Karger, Basel

(124) Greif N (1967). Cutaneous safety of fragrance material as measured by the Maximization test. American Perfumer and Cosmetics 82, 54-57

(125) Hoffmann-LaRoche (1975). Prüfung im 5 Tage Toxizitätsversuch an Ratten, unpublished report, 10 Nov. 1975

(126) BASF AG, Product Safety, Summary of results, beta-Ionon, Range finding study in Wistar rats, administration in the diet for 4 weeks, unpublished report, project no. 30S0449/02045, 02/449-1, 06 May 2003

(127) BASF AG, Product Safety, beta-Ionon R - Subchronic toxicity study in Wistar rats, administration in the diet for 3 months, unpublished report, project no. 50S0449/02057, 02/449-1, 04 June 2004

(128) Oser BL, Carson S and Oser M (1965). Toxicological tests on flavouring matters, Fd. Cosmet. Toxicol. 3, 563-569

(129) Baer F and Griepentrog F (1967). Die Situation in der gesundheitlichen Beurteilung der Aromatisierungsmittel fuer Lebensmittel, Medizin und Ernaehrung 8, 244-251

(130) Hagan EC et al. (1967). Food flavourings and compounds of related structure: II. Subacute and chronic toxicity. Food Cosmet. Toxicol., 5, 141-157, cited in Toxicological Evaluation of Certain Food Additives, Joint FAO/WHO Expert Committee on Food Additives, Geneva, WHO Food Additives Series, No. 19, alpha- and beta-ionone, 72-74, 1984

(131) Hagan EC, Hansen WH, Fitzhugh OG, Jenner PM, Jones WI, Taylor JM, Long EL, Nelson AA and Brouwer JB (1967). Food flavourings and compounds of related structure: II. Subacute and chronic toxicity. Fd. Cosmet. Toxicol. 5, 141-157

(132) Ford GP, Butler WH, Hooson S, Gaunt IF and Hawkins IF (1983). The short-term (90 days) toxicity of alpha- and beta-ionone in rats. Unpublished report from the British

Industrial Biological Research Association, cited in WHO Food additives Series No. 19, 72-74, March 1984

(133) Mortelmans K, Haworth S, Lawlor T, Speck W, Tainer B and Zeiger E (1986). Salmonella Mutagenicity Tests: II. Results from the testing of 270 chemicals. Environmental Mutagenesis 8, Suppl. 7, 1-119

(134) Florin I, Rutberg L, Curvall M, and Enzell CR (1980). Screening of tobacco smoke constituents for mutagenicity using the Ames' test. Toxicology 18, 219-232.

(135) Ono Y, Somiya I and Kawamura M (1991). The Evaluation of Genotoxicity using DNA repairing test for chemicals produced in chlorination and ozonation processes. Wat. Sci. Tech. 23, 329-338

(136) Yoo YS (1986). Mutagenic and antimutagenic activities of flavoring agents used in foodstuffs. Journal of Osaka City Medical Center, 34, 267-288.

(137) BASF AG, Product Safety, Cytogenetic study in vivo with beta-Ionon R in the mouse micronucleus test, single intraperitoneal administration, unpublished report, project no. 26M0449/024134, 02/0449-1, 09 December 2003

(138) Shamberger RJ (1971). Inhibitory effect of vitamin A on carcinogenesis, J. Nat. Cancer Inst. 47, 667-673

(139) BASF AG, Product Safety, Summary of results, Beta-Ionon R, Maternal toxicity study in Wistar rats (range finding), oral administration (gavage), unpublished report, project no. 10R0449/02050, 02/449-1, 12 May 2004

(140) BASF AG, Product Safety, beta-Ionon R - Prenatal developmental toxicity study in Wistar rats, oral administration (gavage), unpublished report, project no. 30R0449/02056, 02/449-1, 19 May 2004

(141) Gomes-Carneiro MR, De-Oliveira ACAX, De-Carvalho RR, Araujo IB, Souza CAM, Kuriyama SN and Paumgartten FJR (2003). Inhibition of cyclophosphamide-induced teratogenesis by beta-ionone, Toxicology Letters 138, 205-213

(142) Willhite CC (1986). Structure-activity relationships of retinoids in developmental toxicology. II. Influence of the polyene chain of the Vitamin A molecule, Toxicol. Appl. Pharmacol. 83, 563-575

(143) Tsutsumi S, Yamaguchi T, Komatsu S and Tamura S (1968). On the teratogenic effects of vitamin A-like substances, Congen. Anomal. Res. Assoc. Jap. Proc. 9, 27 (abstract)

(144) Yamamoto Y, Tamura S and Komatsu S (1971). Research of teratogenic action caused by vitamin A. Shika Gakuho 71, 2075-2081

(145) Katz A (1946). Cited in: Opdyke DLJ (1979). Monographs on fragrance raw materials, Ionone, 444-445

(146) Greif N (1967). Cutaneous safety of fragrance material as measured by the Maximization Test, Am. Perfumer Cosmet. 82, 54-57

(147) Mendelsohn HV (1946). Lemon grass oil, Archs. Derm. Syph., 53, 94-98

(148) Buchbauer G, Jirovetz L, Jaeger W, Plank C and Dietrich H (1993). Fragrance compounds and essential oils with sedative effects up on inhalation, J. Pharmaceutical Sci. 82, 660-664

(149) Wenzel DG and Ross CR (1957). Central Stimulating properties of some terpenones. J. Am. Pharm. Assoc. 46, 77-82

(150) Isakov N (1988). Regulation of T-cell-derived protein kinase C activity by vitamin A derivatives, Cellular Immunology 115, 288-298

(151) Yu SG, Qureshi A, and Elson, CE (1993). Beta-Ionone suppresses DMBA-initiated carcinogenesis, FASEB J. 7 (3-4) A69

(152) Jeong TC, Kim HJ, Yun CH, Lee SS, Yang KH, Han SS and Roh JK (1995). Induction of liver cytochrome P450 2B1 by beta-ionone in Sprague Dawley rats, Biochem. Biophy. Res. Commun. 216, 198-202

(153) Suzuki T, Ohtaguchi K and Koide K (1994). Modelling the cytotoxicity of antioxidants to mouse leukaemia L5178Y cells in vitro, Pharmaceut. Sci. Commun. 4, 171-181

(154) Gu HK, Jeong TC, Chun YJ, Yun CH, Roh JK and Choi IK (1997). Induction of cytochrome P450 by ionones in liver lobes of Sprague Dawley rats, Korean J. Toxicol. 13, 4, 385-391

(155) Jeong TC, Gu HK, Park J-I, Yun H-I, Kim H-C, Ha C-S and Roh JK, (1999). Pretreatment of male BALB/c mice with beta-ionone potentiates thioacetamide-induced hepatotoxicity, Toxicology Letters 105, 39-46

(156) Jeong TC, Gu HK, Chun YJ, Yun CH, Han SS and Roh JK (1998). Effects of beta-ionone on the expression of cytochrome P450s and NADPH-cytochrome P450 reductase in Sprague-Dawley rats, Chemico-Biological Interactions 114(1-2), 97-107

(157) Aoki K, Takimoto M, Ota H and Yoshida T (2000). Participation of CYP2A in cocaine-induced heptatotoxicity in female mice, Pharmacology and Toxicology 87, 26-32

(158) Jeong HG, Chun Y-J, Yun C-H, Moon C-K, Lee H-S, Han SS, Lee E-S, Jeong TC (2002). Induction of cytochrome P450 1A and 2B by alpha- and beta-ionone in Sprague Dawley rats, Arch. Pharm. Res. 25, 197-201

(159) De-Oliveira ACAX, Fidalgo-Neto AA and Paumgartten FJR (1999). In vitro inhibition of liver monooxygenases by beta-ionone, 1,8-cineole, (-)-menthol and terpineol, Toxicology 135, 33-41

(160) He L, Mo H, Hadisusilo S, Qureshi A and Elson CE (1997).

Isoprenoids suppress the growth of murine B16 melanomas in vitro and in vivo, J. Nutr. 127, 668-674

(161) Mo H and Elson CE (1999). Apoptosis and cell-cycle arrest in human and murine tumor cells are initiated by isoprenoids, J. Nutr. 129, 804-813

(162) Robottom-Ferreira AB, Aqunio SR, Queiroga R, Albano RM and Pinto LF (2003). Expression of CYP2a3 mRNA and its regulation by 3-methylcholanthrene, pyrazole, and beta-ionone in rat tissues, Brazilian Journal of Medical and Biological Research 36, 839-844

(163) Siems W, Sommerburg O, Schild L, Augustin W, Langhans CD, Wiswedel I (2002). Beta-carotene cleavage products induce oxidative stress in vitro by impairing mitochondrial respiration FASEB 16, 1289-91

(164) Jeong TC, Jordan SD, Matulka RA, Staulis ED, Kaminski EJ and Holsapple MP (1995). Role of metabolism by esterase and cytochrome P-450 in cocaine-induced suppression of the antibody response, The Journal of Pharmacology and Experimental Therapeutics 272, 407-16

(165) Thelestam M, Curvall M and Enzell CR (1980). Effect of tobacco smoke compounds on the plasma membrane of cultured human lung fibroblasts, Toxicology 15, 203-217, 1980

(166) Pilotti A, Ancker K, Arrhenius E and Enzell C (1975). Effects of tobacco and tobacco smoke constituents on cell multiplication in vitro, Toxicology, 5, 49-62

(167) Wertz PW, Kensler TW and Mueller GC (1978). Inhibition of phorbol ester action in lymphocytes by 5,6-epoxy-beta-ionone, Journal Biochemical and Biophysical Research Communications 83, 138-143.

(168) Parke DV and Rahman H (1969). The effects of some terpenoids and other dietary anutrients on hepatic drug-metabolizing enzymes, Biochemical Journal 113, 12

(169) Petersson B, Curvall M and Enzell CR (1982). Effects of tobacco smoke compounds on the ciliary activity of the embryo chicken trachea in vitro, Toxicology, 23, 41-55

(170) Petterson B, Curvall M, and CR Enzell (1980). Effects of tobacco smoke compounds on the noradrenaline induced oxidative metabolism in isolated brown fat cells, Toxicology 18, 1-15

(171) Ide H and Toki S (1970). Metabolism of beta-ionone. Isolation, characterization and identification of the metabolites in the urine of rabbits, Biochem. J. 119, 281-287

(172) Nixon JE, Hendrickx JD, Pawlowski NE, Pereira CB, Sinnhuber RO, and Bailey GS (1984). Inhibition of aflatoxin B1 carcinogenesis in rainbow trout by flavone and indole compounds, Carcinogenesis 5, 615-619

(173) Yu SG, Anderson PJ and Elson CE (1995). Efficacy of beta-ionone in the chemoprevention of rat mammary carcinogenesis, J. Agric. Food Chem. 43, 2144-2147

(174) Pinching J and Doving KB (1974). Selective degeneration in the rat olfactory bulb following exposure to different odours, Brain Research 82, 195-204

(175) Flint OP and Brown LP (1987). In CIIT-Series: Approaches to elucidate mechnisms in teratogenesis; Eighth CIIT Conference on Toxicology, Research Triangle Park, April 2-3, 1986 ISBN 0-89116-584-3, 185-196

(176) Toxicological Evaluation of Certain Food Additives (1979). Joint FAO/WHO Expert Committee on Food Additives, Geneva, beta-Ionone, 54-56, 2-11 April 1979

(177) Toxicological Evaluation of Certain Food Additives (1984). Joint FAO/WHO Expert Committee on Food Additives, Geneva, WHO Food Additives Series, No. 19, alpha- and beta-ionone, 72-74, 19-28 March 1984

(178) Opdyke DLJ (1979). Monographs on fragrance raw materials, Pergamon Press, Ionone, pp 444

(179) Munro IC and Kennepohl M (2001). Comparison of estimated daily capita intakes of flavouring substances with no-observed-effect levels from animal studies, Fd. Chem. Toxicol. 39, 331-354

(180) WHO (1999). International Programme on Chemical Safety, Safety evaluation of certain food additives, WHO Food Additives Series 42, Ionones and structurally related substances, prepared by the 51st meeting of the Joint FAO/WHO Expert Committee on Food additives (JECFA)