FOREWORD INTRODUCTION

PSEUDOIONONE

CAS N°: 141–10–6

SIDS Initial Assessment Report

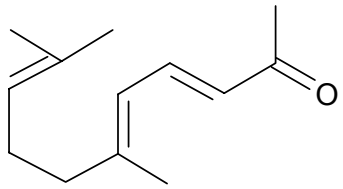
For

SIAM 18

Paris, France, 20–22 April 2004

1.	**Chemical Name:**	Pseudoionone
2.	**CAS Number:**	141–10–6
3.	**Sponsor Country:**	Switzerland
4.	**Shared Partnership with:**	F. Hoffmann-La Roche Ltd
5.	**Roles/Responsibilities of the Partners:**	Industry sponsor: collation of data, preparation of SIDS, RSS, SIAR and SIAP; Sponsor country: review of reports, presentation to SIAM
•	Name of industry sponsor /consortium	F. Hoffmann-La Roche Ltd, Switzerland/ Pseudoionone Consortium with BASF AG, Germany
•	Process used	The documents were written by F. Hoffmann-La Roche Ltd, Switzerland
6.	**Sponsorship History**	
•	How was the chemical or category brought OECD HPV Chemicals Programme?	ICCA Initiative
7.	**Review Process Prior to the SIAM:**	Industry in-house review, then review at the Swiss Federal Office of Public Health and the Swiss Agency for the Environment, Forests and Landscape
8.	**Quality check process:**	Within review process, data in SIAR were compared with data in the SIDS Dossier and for selected endpoints a comparison with original studies was done
9.	**Date of Submission:**	January 21 2004
10.	**Comments:**	

SIDS INITIAL ASSESSMENT PROFILE

CAS No.	141–10–6
Chemical Name	Pseudoionone
Structural Formula	

SUMMARY CONCLUSIONS OF THE SIAR

Human Health

Pseudoionone has an acute oral mammalian LD_{50} (rat and mouse) above 2000 mg/kg bw, with most values greater than 5000 mg/kg bw. The acute dermal LD_{50} (rabbit) is above 5000 mg/kg bw. No inhalative or intraperitoneal toxicity data have been located.

Pseudoionone is severely to moderately irritating to the skin down to concentrations below 10%, based on studies in rabbit and guinea pig, but an 8% solution in petrolatum was not irritating to human volunteers. Pseudoionone produced transient irritant reactions of the eyes in a rabbit study. In a sensitisation test the reactions were judged to be of an irritant rather than a sensitising nature; however, a maximisation test with 8% pseudoionone in human volunteers resulted in 9 out of 108 subjects (8.3%) showing positive reactions.

The 28-day subchronic oral NOAEL of 50 mg/kg bw/d is based on minor, reversible effects (salivation kidney and liver weight gains) up to the highest dose of 1000 mg/kg bw/d. The same effects were observed in a one-generation reprotoxicity study in rat leading to a NOAEL for parental systemic toxicity of 120 mg/kg bw/d and a NOEL of 40 mg/kg bw/d, respectively.

Pseudoionone was not mutagenic in two bacterial Ames tests with and without metabolic activation nor in an *in vivo* OECD 474 mammalian micronucleus test. No carcinogenicity data have been located.

In a one-generation reproductive toxicity study in rats with an average exposure of 60 days for females and of 106 days for males, 120 mg/kg bw/d is the parental systemic toxicity NOAEL based on salivation, kidney and liver weight gains. Development of pups was unaffected up to the highest dose of 360 mg/kg bw/d leading to a developmental NOAEL of 360 mg/kg bw/d. Due to an increased rate in pup deaths during days 1–4 *post partum* in the highest dose group, the reproductive toxicity NOAEL is 120 mg/kg bw/d. A single application by gavage of 960 mg pseudoionone/kg bw to pregnant hamster dams caused no adverse effects on foetal development, in spite of reduced maternal bodyweight gain.

In several *in vitro* or *ex vivo* studies, pseudoionone was shown to have a potential for cytotoxicity at comparatively high concentrations.

In conclusion, the overall mammalian toxicity of pseudoionone is considered to be low. However, based on animal data, pseudoionone is a skin irritant and a weak eye irritant, and based on human data, there is a potential for sensitisation.

Environment

Pseudoionone is a liquid at room temperature, with a melting point of –75 °C, a boiling point of 265.4 °C, vapour pressure of 0.001741 hPa (20 °C), water solubility of 97 mg/l and a $\log P_{OW}$ of 4.0. It has no ionisable groups at environmentally relevant *p*H. Due to the calculated $\log K_{OC}$ values of 2.84 and 3.46 pseudoionone is predicted to

adsorb moderately to organic carbon in soils and sediments. Based on standard Mackay distribution models, pseudoionone will mainly remain and be degraded in the environmental compartment of emission. Pseudoionone has no hydrolysable bonds. When exposed to atmospheric oxygen, pseudoionone is liable to slow autoxidation, but in case of exposure over large surfaces, *e.g.*, on cleaning rags, it may even self-ignite. The total atmospheric half-life due to indirect photodegradation is estimated at approximately 10 minutes. Based on the experimental $\log P_{OW}$ and on QSAR-modelled $\log K_{Ow}$ and BCF values (240-500), pseudoionone has a potential for bioaccumulation.

Pseudoionone attained 62% ready biodegradability in an OECD 301F test but failed the 10-day-window criterion; additional reports support aerobic biodegradability. Pseudoionone was not biodegradable under anaerobic conditions in an ISO 11734 test, being toxic to the sludge at the test concentration of 122 mg/l.

Pseudoionone was moderately toxic in acute aquatic ecotoxicity tests, with EC_{50} and LC_{50} values for freshwater fish, daphnids, green algae and cyanobacteria consistently between 1 and 10 mg/l : *Leuciscus idus*, 96-hour-LC_{50} = 4.64 mg/l, *Daphnia magna*, 48-hour-EC_{50} = 3.7 mg/l and *Scenedesmus subspicatus*, 72-hour-EbC_{50} = 1.11 mg/l respectively ErC_{50} = 2.02 mg/l, all data nominal concentrations. Pseudoionone had low toxicity to activated sludge with an EC_{50} > 1000 mg/l in a 30-minute OECD 209 test, moreover, it was not inhibitory in the ready biodegradability test at 45 mg/l. In contrast, it was toxic to anaerobic sludge bacteria at 122 mg/l and the LOEC to cyanobacteria was 3 mg/l. Based on very summary data for marine larvae and crustaceans, pseudoionone was toxic respectively inhibitory at unspecified low concentrations.

In a chronic and reproductive test with the common soil and sediment nematode *Caenorhabditis elegans* the NOEC of pseudoionone was a relatively high 100 mg/kg sediment (dry weight) for growth and egg production and 400 mg/kg for fertility, while the respective EC_{50} values were 2490, 821 and 1537 mg/kg. Pseudoionone showed juvenile-hormone-like activity in a number of insect species when applied topically at 10–80 µg per larva, which corresponds to a relatively weak effect in comparison with other terpenoids. Pseudoionone was toxic by oral uptake to mosquito larvae with an LC_{50} of 10.15 µg/l diet but it had no effect on honeybees at unspecified concentrations <1% in food. No avian data have been located.

Pseudoionone has been detected in a number of flowering plants and one mould, where it was made likely to be both a precursor and a metabolite of the common carotenoid lycopene. No phytotoxicity data have been located. Some sources show moderate toxicity towards certain fungi, moulds and bacteria, however, these data are difficult to quantify or to relate solely to the activity of pseudoionone.

In conclusion, pseudoionone is not readily biodegradable due to missing the 10-day window criterion, but expected to easily meet the criterion for inherent biodegradability, also based on a test with a closely related substance. Pseudoionone shows moderate toxicity towards aquatic and micro-organisms and low toxicity towards a common soil and sediment nematode. It has weak juvenile-hormone activity in several insects. There is an absence of toxicity studies examining terrestrial plants. However, pseudoionone has been identified as a biochemical intermediate and a metabolite in several plants. On the other hand, there may be some toxicity against fungi and bacteria.

Exposure

In Switzerland approximately 72 % of the produced pseudoionone are used on-site and processed in closed systems. Approximately 26 % are transferred by rail to a plant of the same group in Switzerland and processed in closed systems as well. Less than 1.5 % are shipped in barrels to three other companies. A similar situation applies to the coproducer in Germany.
Worldwide, approximately 40,000 tonnes pseudoionone per annum are estimated by industry to be produced. 99.9% of synthetic pseudoionone is used as an intermediate in the synthesis of vitamins A, E and K_1, of carotenoids and of terpenoid compounds. In addition, pseudoionone appears naturally in plants as an intermediate in the biosynthesis and a metabolite in the degradation of lycopene. Lacking quantitative data, the amount of pseudoionone appearing from natural sources cannot be estimated, but it may be rather high.

Chemical production workers in the two production sites in Switzerland and in Germany and the main recipient companies are rarely exposed to pseudoionone, due to closed synthesis. Where direct contact is possible, *e.g.*, during sampling, filling of transport containers or maintenance work, standard occupational hygiene measures limit exposure. Some of the industrial pseudoionone is released to the atmosphere. Minor amounts are expected in industrial wastewater, no measured environmental concentrations have been located.

Pseudoionone is listed as a food ingredient in the European Union, but not in the United States, hence the public in the EU may be exposed to pseudoionone as an ingredient of food and beverages; while no quantitative data have been located, the actual use in food must be minimal. The use of pseudoionone as a fragrance compound in

cosmetics was forbidden in the EU due to the sensitising potential and pseudoionone is only tolerated as an impurity at less than 2% in pure ionone fragrance compounds, hence exposure through cosmetics must also be minimal.

RECOMMENDATION

The chemical is currently of low priority for further work.

RATIONALE FOR THE RECOMMENDATION AND NATURE OF FURTHER WORK RECOMMENDED

Human Health:

The only hazards identified are irritation to skin and slight irritation to eyes as well as sensitisation. 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.

Environment:

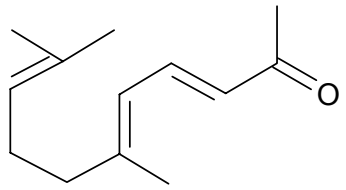
The chemical possesses properties indicating a hazard for the environment. Based on data presented by the Sponsor country, exposure to the environment is anticipated to be low, and therefore this chemical is currently of low priority for further work. Countries may desire to investigate any exposure scenarios that were not presented by the Sponsor.

SIDS Initial Assessment Report

1 IDENTITY

1.1 Identification of the Substance

CAS Number: 141–10–6
IUPAC Name: 6,10-Dimethylundeca-3,5,9-trien-2-one
Molecular Formula: $C_{13}H_{20}O$
Structural Formula:



SMILES Code: CC(=O)C=CC=C(C)CCC=C(C)C
Molecular Weight: 192.30
Synonyms: Pseudo-ionone

2-Pseudoionone

psi-Ionone/ψ-Ionone

Citrylideneacetone

9-apo-psi-Caroten-9-one/9-apo-ψ-Caroten-9-one

3,4-Dehydrogeranylacetone

Pseudoionone is an acyclic C_{13} ketone with a terpenoid skeleton. It is a mixture of cis-pseudoionone (CAS 33073–35–7) and trans-pseudoionone (CAS 3976–54–1) [SciFinder online database, 2003] with a slight preponderance of the cis isomer, due to the method of manufacturing [Roche, technical substance documentation].

Pseudoionone is listed in the following chemical inventories: Australian AICS, Canadian DSL, EU EINECS, Japanese ENCS, Korean ECL, Philippine PICCS, US TSCAand the EU Register of flavouring substances used on or in foddstuffs [Commission of the European Communities, 1999; SciFinder online, 2003; US EPA Chemical Registry System online, 2003].

1.2 Purity/Impurities/Additives

Being to the greatest part (>99.9%, Roche estimate) an intermediate product, the pseudoionone specifications in the reporting company, F. Hoffmann-La Roche Ltd, Switzerland, stipulate a purity for technical pseudoionone (cis + trans isomers) of ≥ 90% w/w by gas chromatography. Typical purities for produced lots are 95–97%.

Impurities comprise mainly two additional pseudoionone isomers ($C_{13}H_{20}O$, sum according to the specifications ≤ 2.5% w/w), 6-methylhept-5-en-2-one (CAS 110–93–0, ≤ 1%), 3,7-dimethyloct-6-

en-1-yn-3-ol (CAS 29171–20–8, ≤ 1.5%), C_{16} components (various isopropylidene-substituted pseudoionone compounds, ≤ 3.5%) and other, undefined impurities (≤ 3.5%) [Specifications, Roche].

Pseudoionone contains no additives.

1.3 Physico-Chemical properties

Table 1 Summary of physico-chemical properties

Property	Value	Reference/comment
Physical state	yellow liquid at room temperature	Specifications, Teranol Lalden
Melting point	–75 °C	Roche, technical substance documentation
Boiling point	265.4 °C	Roche, technical substance documentation
Relative density	0.8951 g/cm^3	Baglay *et al.,* 1988
Vapour pressure	2.8 hPa (109.4 °C) 0.001741 hPa (20 °C)	Baglay *et al.,* 1988 Roche, technical substance documentation
Water solubility	97 mg/l	BASF, internal data.
Partition coefficient n-octanol/water (log value)	3.9/4.1 (25 °C), average = 4.0 average = 4.04	1st/2nd isomer, cis- and trans-pseudoionone, EU A.8 HPLC method; BASF, Caesar & Schäfer, 1989. Average value of 7 QSAR programs; SciFinder 2003, SPARC 2003, VCC-Lab 2003.
Henry's law constant	3.47×10^{-4} to 3.40×10^{-6} atm×m^3/mol	range of 5 calculated Henry's Law constants including experimental vapour pressure divided by experimental water solubility approximation; EPISuite v.3.10, SPARC 2003, EUSES v.1.0
Organic carbon/water partition coefficient, Koc	696 2880	QSAR-calculated, EPISuite v.3.10 QSAR-calculated, SciFinder/ACD Solaris V4.67
Surface tension	32.3 mN/m (20 °C) 27.27 mN/m (20 °C)	Baglay *et al.,* 1988 Roche, technical substance documentation
Viscosity	0.00571 kg/(m×s) (20 °C)	Roche, technical substance documentation
Autoxidation/ Auto-flammability	thin films of pseudo-ionone with large surfaces for air contact are susceptible to autoxidation and even self-ignition	thin film, technical experiment: Finkelshtein & Krasnokutskaya (1996); incident report with pseudoionone-wet cleaning material at Teranol Lalden: F. Hoffmann-La Roche, 2002; similar self-ignition was shown by beta-ionone, Wüest, 1986

Pseudoionone is a liquid at environmentally relevant temperatures. With a low vapour pressure of 0.001741 hPa at 20 °C and a water solubility of 97 mg/l at 25 °C, its calculated Henry's Law constant is also low with ≤ 3.47×10^{-4} atm×m^3/mol. Based on its molecular structure it will not dissociate at any environmentally relevant *p*H value. Pseudoionone has a measured logK_{OW} of 4.0 (mean of the two isomers) and is therefore moderately lipophilic. Moderate sorption to organic carbon in soils or sediment is predicted independently by the EPISuite (2000) model with a K_{OC} of

696 based on the $logK_{OW}$ and by SciFinder (2003)/ACD Solaris with a K_{OC} of 2880 based on molecular conformation and substructure properties. It has a low viscosity of 0.00571 kg/(m×s) at 20 °C and is not particularly surface-active with a surface tension of 27.27–32.3 mN/m at 20 °C.

When present as a thin film forming a large air contact surface, *e.g.,* on cleaning rags, pseudoionone is liable to autoxidation and even self-ignition, probably through ready oxidation of the C=C double bonds, as evidenced by an incident report from the Teranol Lalden production plant. The chemically closely related beta-ionone (CAS 14901–07–6), which has a closed ionone ring in contrast to pseudoionone, shows similar behaviour.

2 GENERAL INFORMATION ON EXPOSURE

Industrial pseudoionone is used almost exclusively (estimate > 99.9%) as a chemical intermediate [Roche, technical substance documentation]. In the Teranol plant at Lalden, Switzerland, pseudoionone is produced in a dedicated, closed system, which is only breached for the following activities: regular sampling (approximately 12 samples per day are taken for analysis through a small sampling port); rare trouble-shooting and technical fault repairs involving opening of the installation; and last, transfer of pseudoionone to transport containers (barrels, lorry tanks, dedicated railway tank transporters) [Hauser, 2002]. A similar dedicated, closed production system is also installed at the BASF production plant in Ludwigshafen, Germany [BASF, pers. comm.].

2.1 Production Volumes and Use Pattern

2.1.1 Chemical Synthesis

According to a crude industry estimate, approximately 40 000 metric tonnes of pseudoionone were reckoned to be produced through chemical synthesis worldwide in the year 2002 [R. Hauser, Teranol AG, Lalden, pers. comm.].

Total chemical synthesis of pseudoionone may start from the addition of acetylene (CAS 74–86–2) to acetone (67–64–1) resulting in 3-methyl-1-butyn-3-ol (115–19–-5), which is hydrated in the presence of a palladium catalyst to 3-methyl-1-buten-3-ol (115–18–4), which is reacted with either diketene or acetic acid ester to the acetoacetate and the latter thermally reacted to 2-methyl-2-hepten-6-one (110–9–0). Alternatively, 3-methyl-1-buten-3-ol is reacted with isopropenyl methyl ether (116–11–0) to 2-methyl-2-hepten-6-one. In a third synthetic pathway, isoprene hydrochloride is reacted with acetone in the presence of an alkaline condensating agent or in the presence of organic bases as catalysts to 2-methyl-2-hepten-6-one. 2-Methyl-2-hepten-6-one is then reacted with acetylene to dehydrolinalool (29171–20–8), to which isopropenyl methyl ether is added to make pseudoionone (141–10–6). Alternatively, 3,7-dimethyl-2,6-octadienal (citral, 5392–40–5; two isomers, citral a = geranial, 141–27–5, and citral b = neral, 106–26–3) is condensed with acetone to pseudoionone [Buttery *et al.,* 1990; Fischer and Löwenberg, 1929; Macek and Vanecek, 1966; Sato *et al.,* 1963; Ullmann's Encyclopedia, 2003]. Pseudoionone may then be further reacted to higher terpenoid compounds, specifically to carotenoids, and also to vitamins E and A.

2.1.2 Use Pattern for Chemically Synthesised Pseudoionone

Pseudoionone is used almost exclusively as a chemical intermediate in the synthesis of vitamins, carotenoids and terpenoid substances [Roche, technical substance documentation]. Approximately 70% of the pseudoionone produced is estimated to be employed in the synthesis of mainly vitamin

E (dl-alpha-tocopherol and its esters) and to a smaller extent of vitamin A (retinyl alcohol and its esters), for use in feed and food fortification and in pharmaceutical specialities. Further, about 25% of the pseudoionone produced is utilised in the synthesis of certain carotenoids, e.g., apocarotene, apocarotenoic ester, beta-carotene, canthaxanthin or lycopene, which are formulated as feed and food additives. The remaining approximately 5% of pseudoionone is reacted in the synthesis of various terpenoid substances, which in turn are mainly used as fragrance but also as flavouring agents.

Use of pseudoionone as an ingredient in public products must be minimal. In the European Union, pseudoionone was banned as a cosmetics ingredient based on published sensitisation data by recommendation of the EU Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers [SCCNFP, 2000a, 2000b]. In this regulation, pseudoionone (and pseudomethylionone) are only tolerated as impurities at an upper limit of 2% in various ionone fragrance substances used for cosmetics [SCCNFP, 2000a, b]. On the other hand, pseudoionone is a registered (and therefore accepted) food flavouring substance in the EU [Commission of the European Communities, 1999], however, actual use data for this application could not be retrieved but based on Roche data this use must be limited to much less than 0.1% of total production.

Based on information from the German SIAM representative in the electronic discussion group, pseudoionone is listed in the Danish SPIN product register for the year 2000/2001.

2.1.3 Natural Occurrence and Formation

Pseudoionone has been identified in extracts from at least 18 different flowering plants [for references please see SIDS chapter 1.11]. These belong mostly to the nightshades (Solanaceae) and the legumes or pulses (Fabaceae) families and include edible (apricot, passionfruit, plum-apricot hybrid, tamarind, tomato) or otherwise consumable species (licorice, tea bush, mate tea, rooibos tea, tobacco). Based on the reports located, pseudoionone is not a rare compound in plant biochemistry, but so far detection seems to be limited to dicotyledonean families.

Mickinney and co-workers [1952] investigated the effect of pseudoionone on carotenoid biosynthesis of the mould *Phycomyces blakesleeanus* (see chapter 4.2, Toxicity to Micro-organisms). At ~ 22 mg pseudoionone/l, growth was nearly normal and overall pigment production was nearly as high as in controls. However, compared to controls chromatography showed a small increase in lycopene, a carotenoid with open terminal rings similar to pseudoionone. In contrast, the biosynthesis of beta-carotene, a lycopene isomer with closed terminal rings, was slightly reduced in the presence of pseudoionone while it was enhanced in the presence of beta-ionone (CAS 14901–07–6), which has a closed ring. The authors conclude that the biosynthesis of lycopene and beta-carotene in *P. blakesleeanus* is markedly influenced by the use and concentration of compounds “presumably providing terminal groups in the carotenoid molecule”. Hence, pseudoionone is highly likely to be a precursor in lycopene biosynthesis.

Conversely, the formation of pseudoionone from lycopene has been demonstrated experimentally through heat-induced physico-chemical degradation of pure all-trans-lycopene by C10–C11 cleavage in the presence of oxygen [Kanasawud & Crouzet, 1990a, 1990b]. In contrast, at low oxygen levels, Kanasawud and Crouzet found no pseudoionone but 2-methyl-2-hepten-6-one instead, showing C6–C7 cleavage of lycopene. Pseudoionone was also confirmed to be formed naturally through degradation or metabolism of higher terpenes respectively carotenoids, probably from lycopene, in the case of the plant *Iochroma gesnerioides* [Alfonso & Kapetanidis, 1994].

In tobacco plants it has long been recognised that mechanical removal of apical (terminal) and axillary (lateral) flower buds, or chemical suppression of their growth, will influence the content of

nicotine and flavour compounds. Weeks and Seltmann [1986] showed that the rate of pseudoionone production also varies. They demonstrated that a combination of removal of the top flower bud and chemical control of all others results in low pseudoionone concentrations while removal of both terminal and axillary buds, without chemical treatment, gives the highest concentration.

The process of curing of plant leaves through microbial fermentation and drying was shown to promote the formation of pseudoionone in the case of Japanese "toyama kurocha" fermented tea, whereas no pseudoionone had been detected in the fresh tea *(Camellia sinensis)* leaves [Kawakami & Shibamoto, 1991]. Along with fermentation, photo-oxidation and auto-oxidation are listed as probable mechanisms of pseudoionone formation. However, subsequent long-term storage of dry "toyama kurocha" tea over one year led to loss of more than half of pseudoionone. In the case of tobacco leaves, where pseudoionone is already present in the green leaves, similar curing strongly enhanced the pseudoionone content [Pilotti *et al.,* 1975; Thelestam *et al.,* 1980; Petterson *et al.,* 1982; Forsblom *et al.,* 1991]. This formation of pseudoionone (among other compounds) was also attributed to degradation of natural carotenoids or terpenoids. Some of the pseudoionone contained in tobacco has also been found in tobacco smoke.

As the amount of pseudoionone formed in tobacco plants is dependent on removal or control of phytohormone-releasing organs (the flower buds), it becomes probable that the natural pseudoionone formed in plants is not only a metabolite but also a precursor of larger-sized compounds. This would make it a relatively common substance in plant biochemistry. However, in spite of the relatively wide distribution in plants, including edible or otherwise consumable species, the natural formation of pseudoionone cannot be reasonably quantified, nor can human exposure to natural pseudoionone through food products or tobacco smoke be estimated.

2.2 Environmental Exposure and Fate

2.2.1 Sources of Environmental Exposure

Due to the closed production system at both Teranol Lalden and BASF Ludwigshafen plants, exposure of the environment to pseudoionone is limited to sampling, maintenance and filling respectively transfer operations plus residual pseudoionone from extraction and solvent recycling [Hauser, 2002; BASF, pers. comm.].

Measured or materials balance data are available from the last step in pseudoionone synthesis, the isopropenyl methyl ether addition to dehydrolinalool [Hauser, 2002]. Combustible distillation residues, mostly solvents, sum up to approximately 23 kg/t pseudoionone. All gaseous emissions within the closed system are collected and incinerated together with the liquid residues and gaseous emissions from other installations in the Teranol Lalden plant as well as spent cleaning solvents in an in-house special waste incinerator. The heat gained is used in production and heating of offices. With an expected standstill of the gas incinerator of <5% of targeted operating time, a maximum of <0.01 kg total gaseous emissions (including pseudoionone) per tonne of pseudoionone produced is reckoned to be lost into the surrounding atmosphere. During opening of the system and container filling, some losses to the atmosphere are unavoidable but the amounts are small due to the low vapour pressure (<0.002 hPa). All filling operations take place under vent hoods that bleed off over the factory roof. Aqueous distillation residues from solvent recycling and product extraction contain a measured 5.8 kg total organic carbon/t pseudoionone. This wastewater showed ≥98% biodegradability in an inherent test [Roche, internal data], it is treated with other aqueous waste streams in the combined municipal-industrial sewage works before release into receiving waters, the river Rhone in the case of the Teranol Lalden plant. Due to tarmac-sealed industrial surfaces with collection of

rainwater (and also spills and fire-fighting waters), no direct emission into industrial or other soil is expected.

Comparable installations and substance losses to distillation residues, gaseous emissons or wastewater apply for the BASF Ludwigshafen plant in Germany, where waste treatments also correspond to those described above [BASF, pers. comm.]. Hence, from two major European pseudoionone production plants, no major emissions into the environment are expected.

Due to lack of data, the additional environmental exposure through natural plant-derived pseudoionone cannot be estimated.

2.2.2 Photodegradation/Atmospheric degradation

No experimental data have been data located. QSAR modelling with EPISuite v.3.10 predicts an overall hydroxyl-radical-mediated atmospheric degradation half-life of 29.5 minutes and an ozone-mediated half-life of 12.2 minutes; it further notes that reaction with nitrate radicals may be important. The total atmospheric half-life of pseudoionone is estimated by EPISuite at 10.2 minutes. Based on this QSAR model, pseudoionone is expected to degrade rapidly in the atmosphere.

2.2.3 Stability in Water

No data have been located. Based on the molecular structure, hydrolysis can be excluded.

2.2.4 Stability in Other Media

Kawakami and Shibamoto [1991] reported that the concentration of pseudoionone in dry (10–13% water) “toyama kurocha” fermented tea decreased by more than half during storage of over one year. This is interpreted as evidence that pseudoionone will degrade even in a very dry environment, probably through autoxidation with atmospheric oxygen, which in the extreme form of autoflammability has also been shown experimentally [Finkelshtein & Krasnokutskaya, 1996] and in an industry incident report [F. Hoffmann-La Roche, 2002].

2.2.5 Transport between Environmental Compartments

Static (Mackay Level I) and dynamic (Mackay Level III) fugacity-driven distribution models [EQC, 2003] were run with the available and modelled basic data for pseudoionone. In the static Level I model (a single emission both air, water and soil; no degradation; no advection; unlimited time for equilibrium distribution), pseudoionone is predicted to distribute mainly (87% of mass) to soil, while 10% are expected in water, a further 2% in sediment, 0.7% in air, 0.07% adsorbed onto suspended particles and 0.005% in fish.

In the dynamic Level III model (permanent emission into one or more compartments, degradation half-lives adapted from experimental aerobic and anaerobic biodegradability results and from EPISuite v.3.10, advection, determination of statistical residence time in model system), the following dynamic distributions were predicted. The Level III model was run with emissions exclusively into one of the four standard compartments and additionally with equal emissions to air and water, as might be expected in more realistic circumstances, based on data from the Teranol Lalden production plant (some losses into the air during opening of the closed production system for maintenance, some indirect losses from solvent distillation or cleaning into industrial wastewater).

Table 2 Dynamic (Mackay Level III) distribution

Compartment, Residence time	Emission to compartment				
	100% to air	100% to water	100% to soil	100% to sediment	50% to air, 50% to water
Air, %	70.4	0.000542	0.000016	<0.00001	0.0689
Water, %	9.1	64.6	0.0601	1.01	64.2
Soil, %	15.5	0.000119	99.9	<0.000002	0.0152
Sediment, %	4.98	35.3	0.0329	99.0	35.3
Residence time, h	0.343	353	300	15641	177

The single-compartment-emission modelling shows that pseudoionone will mainly remain in the compartment of emission: In the air, abiotic atmospheric degradation is predicted to proceed so quickly that pseudoionone will not have sufficient time to partition to a major part to other compartments, with a resulting short overall residence time of about 20 min. In water, there is a major distributon of approximately one-third to sediment, with a longer residence time of 353 h due to the negligible anaerobic biodegradability (t½ set at 10×E+11 h, default for negligible degradation) in the sediment. In both soil and sediment compartments, at least 99% of the total mass is expected to remain in the respective compartment; however, residence time is predicted to be relatively low (300 h) in case of emissions only to soil, due to appreciable aerobic biodegradability, while in the sediment a very long residence time (15641 h, nearly 2 years) is expected, due to the anaerobic virtual non-biodegradability.

In the "more realistic" model run with emissions to both air and water, the distribution is comparable to the water-only scenario, with the notable exception that with 177 h the overall residence time is half that of the water scenario, due to predicted rapid atmospheric degradation.

Based on both static and dynamic modelling, the sediment is identified as the only compartment of potential concern regarding persistence. However, predicted residence times are limited (< 15 d) as long as the original emission into the system is not exclusively into the sediment. Only under the latter, highly unrealistic, assumption would pseudoionone show persistence. In all other scenarios the anaerobic non-degradability would be compensated by relative mobility due to only moderate adsorption, also in the sediment, resulting in advection out of the system.

2.2.6 Biodegradation

One standard aerobic and anaerobic biodegradation test each has been located for pseudoionone, as well as additional information based on non-standard studies and an inherent biodegradation test with a closely related test substance.

Table 3 Summary of biodegradation data

Test	Result	Reference/comment
Ready biodegradability, Manometric respirometry test, OECD 301F	62%, 27 d	not readily biodegradable due to missing the 10-day-window criterion; initial lag phase of 7 d; Pagga, 2002, reprint of original BASF test report from 1988
Primary biodegradation by two fungi, *Aphanocladium album* and *Rhodotorula mucilaginosa*	100% primary degradation, 14 d	the two fungi tested are capable of complete primary degradation of pseudoionone within 14 d; three metabolites were identified; Dmochowska-Gladisz *et al.,* 1987
Ultimate anaerobic biodegradability, ISO 11734	0%, 93 d	pseudoionone is not anaerobically biodegradable; inorganic carbon production compared with the inoculum blank showed toxicity/inhibition; Häner, 2002
Inherent biodegradability, OECD 302C, with *test substance beta-ionone*	97%, 28 d	the closely related substance, beta-ionone, was largely degraded in a standard inherent test; Gröner, 1989

In a manometric respirometry ready biodegradability test according to OECD 301F [Pagga, 2002a] with a pseudoionone concentration of 45 mg/l, there was an initial lag phase and degradation really started only on day 7 (5% BOD/ThOD). On day 8, 32% BOD/ThOD showed rapid degradation, which, however, already started to slacken in the following days. 50% was reached on day 17 and a plateau of 61% on day 25. By day 28, degradation remained at 62%. Hence, pseudoionone was well aerobically degradable but did not attain ready biodegradability due to the 10-day-window criterion.

Primary biodegradation of pseudoionone by two species of fungi was described by Dmochowska-Gladisz and colleagues in 1987. Both *Aphanocladium album* (Hyphomycetes; an insect parasite) and *Rhodotorula mucilaginosa* (Basidiomycetes; a soil fungus) achieved 100% primary degradation in a submerged culture system with an initial concentration of 120 mg pseudoionone/l within 14 days. Metabolites of pseudoionone were identified. After 14 days' incubation the *A. album* medium contained 45 mg/l of (+)-6,10-dimethyl-5,9-undecadien-2-ol (CAS 50373–44–9) as the main metabolite, while the *R. mucilaginosa* culture resulted in 30 mg/l of 6,10-dimethyl-5,9-undecadien-2-one (CAS 689–67–8) and 8 mg/l of (–)-6,10-dimethyl-5,9-undecadien-2-ol (CAS 116048–77–2). Other degradation products remained unidentified due to low chloroform extractability and relatively high volatility. Still, the experiments showed that two fungi from different groups were able to degrade pseudoionone by hydrogenation of the C3 double bond with subsequent reaction of the carbonyl group. The two fungi differed, however, in the optical rotation and relative amount of products.

An inherent biodegradability test was run with the chemically closely related substance beta-ionone (CAS 14901–07–6) at 30 mg/l according to OECD 302C [Gröner, 1989], run with activated sludges from a municipal sewage works and and industrial in-house monitoring pilot sewage treatment plant. At the end of the test (28 d), fully 97% of beta-ionone was degraded as measured by BOD. This is taken as supportive evidence for a high aerobic biodegradability of pseudoionone.

An ultimate anaerobic biodegradation test with pseudoionone according to ISO 11734 was performed by BMG Laboratories operating under SN EN 45001 quality assurance system [Häner, 2002]. In an anoxic sludge system with three pseudoionone test flasks, three inoculum blank flasks and two positive control flasks, the gaseous inorganic carbon (IC; methane and carbon dioxide) in the headspace was determined regularly and the IC in the liquid phase was measured at the end of the test. The positive control, diethylene glycol, reached a degradation rate of 82% (measured net IC/ theoretical IC) at 41 days and thereby confirmed the viability of the sludge system. In contrast, at an initial concentration of 122 mg/l, pseudoionone was not anaerobically degraded at all. Moreover,

after substraction of the blank control IC for the same sampling points, the calculated net pseudoionone IC production was consistently negative, showing inhibition of respectively toxicity to the anaerobic bacteria at the concentration used. In line with the guideline, anaerobic biodegradability has not been tested at lower concentrations.

In conclusion, pseudoionone is not readily biodegradable due to missing the 10-day-window criterion. It was degraded by aerobic micro-organisms, both in a ready biodegradation test with activated sludge and in primary degradation experiments with two species of fungi. However, it was not degradable at all in an ultimate anaerobic degradation test, but was toxic to the anaerobic bacteria at a concentration of 122 mg/l.

The behaviour and fate of pseudoionone in sewage works was modelled using various models. Entering basic physico-chemical properties and the nearly ready biodegradability (except for the 10-day-window criterion), respectively the corresponding parameters according to the documentation or help for the respective programs, the following predictions in per cent of influent were derived.

Table 4 Fate in sewage works models

	Percentages according to models				
	STP v.1.50[a]	**STP/EPISuite v.3.1[a]**	**SimpleTreat v3.0[b]**	**SimpleTreat v3.0[c]**	**EUSES[d]**
Influent	100	100	100	100	100
Sludge adsorption	15.2	5.9	18.4	16.7	17.3
Biodegradation	80.1	92.2	41.0	64.1	55.2
Volatilisation	0.02	0.0	0.2	0.1	0.2
Total removal	**95.2**	**98.1**	**59.6**	**81.0**	**72.7**
Effluent	4.8	1.9	40.4	19.0	27.3

a) Settings: half-life in primary settler = 30 h, in aeration and final settler = 3 h.
b) Biodegradation constant k = 0.3/h (EU Technical Guidance Document default for ready biodegradability), with primary sedimentation.
c) Biodegradation constant k = 1.0/h (upper SimpleTreat limit), with primary sedimentation.
d) Selected "readily biodegradable, failed 10-day-window".

All models predict appreciable (~60%) to very high (~98%) removal of pseudoionone in a sewage works, which is expected to result mainly (69–94% of total removal) from biodegradation. In all cases, volatilisation is negligible, while 5.9–18.4% is forecast to adsorb to activated sludge. Depending on the model, 2–40% of pseudoionone is expected to pass unchanged through a treatment plant. The lowest removal rate and highest emission in effluent are predicted by SimpleTreat using the conservative EU Technical Guidance Document [Commission of the European Communities, 1996] degradation defaults.

2.2.7 Bioaccumulation

No experimental data have been located. Four different QSAR-calculated bioconcentration factors for pseudoionone range from 239.9 to 501 [EUSES, 1997; EPISuite v.3.10, 2000; ChemSCORER, 2002; SciFinder, 2003], in agreement with a measured n-octanol/water partition coefficient of 10 000 [$\log K_{OW} = 4$; Caesar & Schäfer, 1989].

A recent chemical scoring and ranking QSAR software developed by the Canadian group of Don Mackay [ChemSCORER, 2003] predicts an overall bioaccumulation factor of 1647 for lake trout, comprising both biomagnification through a sediment and water foodnet and bioconcentration from water, based on physico-chemical basic data for pseudoionone.

In conclusion, based on a measured $\log K_{OW}$ of 4.0 and on QSAR-calculated bioconcentration and bioaccumulation factors, in the absence of empirical data, pseudoionone is predicted to have a potential for bioconcentration [$\log K_{OW} \geq 4$; OECD, 2001, p. 73], respectively to have a tendency for moderate bioconcentration [$100 \leq BCF < 1000$; Smrchek, 2000] .

2.2.8 Other Information on Environmental Fate

Synthesised pseudoionone is expected to be emitted to the environment in small amounts and low concentrations, into the air or into wastewater streams. In the atmosphere, rapid indirect photo-degradation mediated by hydroxyl and nitrate radicals or ozone is anticipated. Based on various degradation tests, pseudoionone is predicted to biodegrade rapidly in aerobic surroundings, in sewage works, but also in surface waters, soil and seawater. Additionally, physico-chemical oxidation is expected to be an important degradation pathway in all aerobic environmental compartments. Only in an anaerobic environment, *e.g.,* in sewage sludge digesters and in deeper sediment layers, is pseudoionone expected to remain chemically stable for a longer time.

Due to the appreciable water solubility and a moderately high octanol/water partition coefficient, respectively a moderate calculated adsorption coefficient, pseudoionone is expected to retain some environmental mobility. Substance adsorbed to sediment may be re-mobilised and enter aerobic compartments again, where physico-chemical or biodegradation is expected. With the possible exception of sediment, no high or persistent concentrations of pseudoionone are predicted in the environment.

No measured environmental concentrations for pseudoionone have been located.

2.3 Human Exposure

2.3.1 Occupational Exposure

Due to closed production systems at the Teranol Lalden plant and in view of forced air-changes in the chemical production buildings, exposure of technicians to pseudoionone during regular operations is negligible.

Minimal exposure is possible during inspection, cleaning or repair work, however, the installation is vented and flushed with air before any work within. Again, minimal exposure is possible during sample taking and transfer of pseudoionone to other containers for transport. All filling operations take place under vent hoods that bleed off over the factory roof. Due to (forced) aeration and low vapour pressure of pseudoionone, exposure to vapours or aerosols is negligible.

Workers are required to wear solid workclothes with long sleeves, safety shoes, nitrile caoutchouk protective gloves and safety glasses. Hence, during filling or other work on the pseudoionone installation, the technicians are protected against any possible splashes. In the analytical laboratory, lab coats and safety glasses are prescribed.

In confirmation of the above, during more than 30 years of pseudoionone production at the Teranol Lalden plant, no adverse occupational health effects on staff have become known [Hauser, 2002].

2.3.2 Consumer Exposure

Well over 99% of industrially produced pseudoionone is used as a chemical intermediate. Potential consumer exposure to this major part of synthetic pseudoionone is limited to residual, non-reacted substance. This is only expected in few cases where the next product, for which pseudoionone is the direct precursor, is used as a fragrance ingredient. In such cases, the pseudoionone content is limited in the EU and also by the fragrance institute IFRA to a maximum of 2% as an impurity in ionones. In the Fragrance Raw Materials Monograph for pseudoionone, Ford and colleagues [1988] cite "a reported [however, no source given] maximum concentration of 0.8% in consumer products".

Further products derived from pseudoionone are terpenoids, carotenoids and (mainly) vitamins E and A. With exception of a few ionones (above) these are not direct reaction products of pseudoionone and any potential residual pseudoionone will decrease at every single reaction step. Therefore, consumer exposure to pseudoionone as an impurity in synthetic carotenoids or vitamins E and A is negligible.

Pseudoionone is a listed flavour ingredient in the EU, however, no use data have been located nor can an exposure estimate be made. As no information on the specific use of pseudoionone for flavouring purposes is available, no potential concentration or exposure range can be estimated; only a theoretical upper limit can be given in the form of clearly less than 0.1% of an estimated worldwide production of 40 000 t/a, corresponding to less than 40 t/a for the whole world.

In view of the reported presence of natural pseudoionone in commonly used plant products, *e.g.,* tomatoes, apricots, passionfruit, tamarind, Japanese "toyama kurocha" tea, maté tea, rooibos tea and tobacco smoke, consumer exposure to natural pseudoionone may be much more important than to synthetic product, but again this exposure is impossible to quantify.

3 HUMAN HEALTH HAZARDS

3.1 Effects on Human Health

3.1.1 Toxicokinetics, Metabolism and Distribution

No data on toxicokinetics, metabolism or distribution have been located for pseudoionone.

However, in two GLP studies with longer exposure of rats to pseudoionone, an OECD 407 28-day subchronic oral toxicity test [Strobel & Lambert, 1997; see chapter 3.1.5] and an OECD 415 one-generation reproductive toxicity test with mean exposures of 106 days in males and 60 days in females [Beekhuizen, 2003; see chapter 3.1.8], there was a consistent increase in absolute and relative liver and kidney weights, particularly in the males, at the higher doses (250 and 1000 mg/kg bw/d in the OECD 407; 120 and 360 mg/kg bw/d in the OECD 415). In both tests, even in the highest-dose group (1000 respectively 360 mg/kg bw/d), this increase in relative organ weight was not related to any histopathological abnormalities. Moreover, in the 28-day test where half of the high-group animals were kept for an additional treatment-free period, the weight-gain findings had disappeared after the additional 14 days. Hence, increased absolute and relative liver and kidney weight was interpreted as a physiological adaptation to an enhanced metabolic load.

Based on these findings, it is expected that pseudoionone, at least to a major part, is metabolised in the liver and the metabolites excreted by renal pathway.

3.1.2 Acute Toxicity

Acute dermal and oral toxicity studies with pseudoionone date back to the 1970s and 1980s, reflecting the long time that pseudoionone has been produced by industry. Due to the age of these studies, none of them has been conducted under GLP.

Studies in Animals

Inhalative

No inhalative data have been located for pseudoionone. However, in view of the low vapour pressure (0.001741 hPa at 20 °C) and of the closed production systems, there is little probability of major exposure to gaseous pseudoionone. Additionally, as it is not sprayed during further synthesis, no relevant exposure to aerosol-bound pseudoionone is expected, either. Therefore, inhalative exposure to and toxicity of pseudoionone is considered a negligible potential hazard.

Dermal

The dermal toxicity of pseudoionone was investigated in a short report by Moreno [1976] commissioned by the Research Institute for Fragrance Materials (RIFM; Hackensack, NJ, USA). In this limit test, 10 healthy albino rabbits received one dermal application of 5000 mg pseudoionone/kg bw to clipped, intact or abraded abdominal skin under occluded patches for 24 hours of contact. Observations for mortality and/or systemic effects were made during the following 14 days. The dermal reactions were scored on day 1, 7 and 14 after application using the Draize scoring system. The test animals were killed on day 14 after application and a gross necropsy was carried out on all of them. One animal out of 10 is reported dead (time after application not stated) during the test period, all others survived. No clinical signs nor specific findings at necropsy are mentioned in the report. Based on the result of 1/10 dead, pseudoionone has a dermal LD_{50} > 5000 mg/kg bw.

Oral

Table 5 Summary of acute oral toxicity data

Test	Result	Reference/comment
Corresponding to EEC/OECD limit dose method, rat	LD_{50} > 2000 mg/kg bw NOEL = 2000 mg/kg bw	5 males and 5 females, one dose level, no toxic effects were noted nor any deaths after 14 d; BASF test report; Kirsch & Kersebohm, 1988
Roche gavage oral toxicity test, rat	LD_{50} > 8000 mg/kg bw LD_0 = 8000 mg/kg bw	rats of former Roche inbred strain were used in groups of 5 or 10, dose levels were 1000, 2000, 4000 and 8000 mg/kg bw, no deaths after 10 d; Roche test report; Bächtold, 1973a, report no. 4921
Gavage oral toxicity test, rat	LD_{50} >5000 mg/kg bw LD_0 = 5000 mg/kg bw	in a very short report, undescribed rats were used in at least one group of 10, probably one dose level of 5000 mg/kg bw, no deaths after unstated time; RIFM test report; Moreno, 1976
Former Roche gavage oral toxicity test, mouse	LD_{50} = 7270 mg/kg bw LD_0 = 4000 mg/kg bw	rats of former Roche inbred strain were used in groups of 5 or 10, dose levels were 1000, 2000, 4000 and 8000 mg/kg bw, 80% dead at 8000, no deaths at 4000 after 1 d; Roche test report; Bächtold, 1973b, report no. 4748
OECD 473 micronucleus *in vivo* mutagenicity test, mouse, GLP	LD_{50} > 2000 mg/kg bw LD_0 = 2000 mg/kg bw	all of 16 mice (3 females and 3 males in the range-finding test, 5 and 5 males in the two test groups in the main test) survived an oral dose of 2000 mg/kg bw in maize/corn oil for at least 24 up to 72 hours; no toxic effects were noted during regular observations; Buskens, 2003

Four older gavage tests were located from the 1970s and 1980s, three with rats and one with mice; pseudoionone has a low acute oral toxicity to rodents with LD_0 values of 2000, 5000 and 8000 mg/kg bw for rats, an LD_0 of 4000 mg/kg bw for mice and an interpolated LD_{50} of 7270 mg/kg bw for mice [Bächtold, 1973a, 1973b; Moreno, 1976; Kirsch & Kersebohm, 1988]. While none of these tests was performed under GLP and while the reports are short, all of them were conducted in professional industry toxicology laboratories, where regular serial testing in a dedicated facility made for reliable animal keeping, tests substance administration, laboratory protocols and reporting.

Specifically, while still being short, the most recent of these reports [Kirsch & Kersebohm, 1988] is detailed and describes a limit dose test at the regulatory limit of 2000 mg/kg bw with male and female Wistar rats. Supplier, acclimatisation, animal husbandry and test procedures are described, as are the test substance formulation with 0.5% aqueous carboxymethyl cellulose, the dosing, observations, killing and gross necropsy at the end. All test animals survived the dose of 2000 mg/kg bw without any signs recorded during observation or necropsy.

In confirmation of the above results, in the range-finding and main test of a GLP micronucleus test according to OECD 473 [Buskens, 2003], all of 3 females and 3 males in the range-finder and 5 and 5 males in the two main test groups survived a single oral dose by gavage of 2000 mg pseudoionone/kg bw for the duration of the respective tests, 24, 48 or 72 hours. Supplier, acclimatisation, animal husbandry and test procedures are described, as are the test substance formulation with maize/corn oil, the dosing and observations. Specifically, no toxic signs or effects were noted during regular observations.

Based on these five reports, pseudoionone has a low acute oral toxicity in rats and mice with all NOEL respectively LC_0 values consistently at or above (and in three out of the four tested concentration ranges well above) 2000 mg/kg bw.

Studies in Humans

No studies with or data for pseudoionone have been located.

Conclusion

Pseudoionone consistently showed low acute toxicity to rodents in four oral tests and one dermal test located. The oral LD_{50} values ranged from > 2000 up to > 8000 mg/kg bw, the dermal LD_{50} was > 5000 mg/kg bw. No inhalative toxicity data have been located.

3.1.3 Irritation

Skin Irritation

Studies in Animals

One proper skin irritation test [Hildebrand & Kirsch, 1990a] and corroborating data from a GLP dermal sensitisation study [Csato & Chubb, 1996] and an acute dermal toxicity test [Moreno, 1976] with pseudoionone have been located.

In the OECD 404 semi-occlusive dermal irritation test, Hildebrand and Kirsch [1990a] describe the application of 0.5 ml undiluted pseudoionone via patches to the clipped skin of two male and one female White Vienna rabbits. Full details as to supplier, acclimatisation, animal husbandry, test procedures and observations with grading of skin reactions until 15 days after application are given. The single readings are listed in the SIDS. The mean skin reaction values for the three animals, averaged from the readings at 24, 48 and 72 hours, are as follows: animal 1, erythema 3.0, oedema 1.7; animal 2, erythema 3.0, oedema 2.3; animal 3, erythema 2.7, oedema 0.3. The overall average

values for all three animals are erythema 2.9, oedema 1.4. The skin reactions, in particular the erythemata, did not fully resolve until the end of the observation period at 15 days. Therefore, undiluted pseudoionone applied to rabbit skin under semi-occlusive conditions resulted in moderate to severe erythema with a primary irritation index of 2.9 that was slow to resolve; oedematous reactions were weaker but also pronounced at 48 and 72 hours. Pseudoionone is a skin irritant.

In an OECD 406 dermal sensitisation test with guinea pigs under GLP, Csato and Chubb [1996] reported that in a pre-test, an intradermal injection of 0.1-ml aliquots of diluted pseudoionone produced moderately irritant reactions at concentrations of 0.5-5%, while all higher concentrations resulted in severe irritation or tissue necrosis.

In the same study [Csato and Chubb, 1996], a topical second induction of 50% pseudoionone by occlusive application for 24 hours in the main test resulted in "severe" skin responses as evidenced by behaviour and general condition of the animals. The test was aborted and a second topical skin irritation ranging study was conducted with four animals. This ranger finder showed in all animals an intense brown staining of the skin that was related to the test article concentration and which prevented a full scoring of skin reactions; in an additional animal with lower concentrations applied, 6.25% pseudoionone in water was the highest concentration that produced moderate irritation in the short term which would resolve within a few days. In the subsequent main test, however, staining reactions with 6.25% pseudoionone in water were still so strong as to prevent grading of skin reactions. A second challenge with 3.125% and 1.563% pseudoionone in water resulted in weak staining that did not preclude gradings. None of the animals in this second challenge showed any skin responses. Csato and Chubb concluded that higher concentrations (> 10%) of pseudoionone applied topically under occlusion to guinea pig skin produced clear to severe irritation, that the application of 6.25% pseudoionone could not be graded due to staining and that concentrations of ≤ 3.125% pseudoionone in water did not produce any irritant reactions.

In the acute dermal toxicity test [Moreno, 1976; cited in Ford *et al.,* 1988], undiluted pseudoionone was applied to the skin of 10 rabbits using occlusive patches for 24 hours and dermal reactions were scored on days 1, 7 and 14. In the short report, pseudoionone is stated to have "produced moderate irritant effects", which, however, were not otherwise described nor were any scores reported.

Based on two detailed studies, undiluted pseudoionone is severely irritating to skin and all concentrations > 10% produced clear to severe irritation; only concentrations ≤ 3.125% in water did not produce any irritant reactions. In one divergent study, undiluted pseudoionone was reported to be a moderate irritant. Pseudoionone applied by intradermal injection produced irritant reactions at all concentrations of 0.5% and higher.

Studies in Humans

Ford and colleagues [1988] refer to two maximisation test series performed on behalf of RIFM with a total of four human volunteer cohorts (Epstein, 1978; Kligman, 1976; both reports not published), stating that a "48-hr closed-patch test at a concentration of 8% in petrolatum on the forearms or backs of 108 volunteers produced no irritation". However, no further information is contained in the short abstract by Ford and co-workers. Further, no toxic effects hinting at irritation have been reported during many years of occupational handling [Hauser, 2002].

Eye Irritation

Studies in Animals

Two eye irritation test reports for pseudoionone were located, one original report from industry [Hildebrand & Kirsch, 1990b] and, with high probability, the same test cited in an overview for

validation of *in vitro* alternatives to eye irritation tests [Spielmann et al., 1996]. The latter source, however, also contains results from two alternative *in vitro* tests.

In an OECD 405 test without information on GLP, Hildebrand and Kirsch [1990] applied 0.1 ml of undiluted pseudoionone each to the conjunctival sac of the right eyelid of three wite Vienna rabbits. Full details as to supplier, acclimatisation, animal husbandry, test procedures and observations with grading of ocular reactions until 8 days after application are given. Single readings at 1, 24, 48, 72 and 192 hours after application regarding degree of corneal opacity, corneal area involved, iris score, conjunctival redness, conjunctival chymosis and discharge are given, as are the average values per animal and the overall average values for all animals at 24, 48 and 72 hours (please see SIDS for full details). In the latter overall scores, mean corneal opacity was 0.1, the iris score 0.0, conjunctival redness 1.7 and conjunctival chymosis 0.4. The administration of 0.1 ml undiluted pseudoionone caused mainly well-defined conjunctival reddening and transient slight chymosis. Most findings had resolved within 72 hours, none were detected after 8 days. Pseudoionone was slightly irritating to the eye but without long-term damage or effects.

Spielmann and colleagues [1996] also reported an OECD 405 test with pseudoionone, again without any information regarding GLP. They compared experimental data on various test substances with two proposed *in vitro* alternatives to Draize-type eye tests, the Hen's Egg Chorio-Allantoic Membrane (HET-CAM) and the mammalian 3T3 Cell Neutral Red Uptake (3T3 NRU) test. They state that the experimental data were from tests according to OECD 405 performed by "chemical and pharmaceutical companies". The scores given are identical to the ones from Hildebrand and Kirsch [1990] listed above, albeit presented slightly differently. With high probability, Spielmann and coworkers cite the earlier industry study, and derive the same conclusion from it (slightly irritating, transient effects).

Regarding the *in vitro* studies, in the appendix of the paper by Spielmann and colleagues [1990], the test substance 2-pseudoionone is characterised as not labelled according to EC criteria, meaning not irritant to the eye, both by Draize test and by *in vitro* studies. Specifically, 2-pseudoionone is listed to have an irritation threshold > 100%, meaning no irritation, in the HET-CAM test according to data supplied by Henkel KgaA, Germany and the German Bundesgesundheitsamt (Federal Health Office). In the 3T3 NRU test, the IC_{50} for 2-pseudoionone was a low 0.08 mg/ml in the Henkel test and 0.04 mg/ml in the Bundesgesundheitsamt test. Based on these summary data, both the company Henkel KgaA and the Bundesgesundheitsamt had performed alternative tests and found negative results for pseudoionone.

Conclusion

Pseudoionone was moderately to severely irritating to skin in all animals when tested at concentrations > 10%; at a concentration of 6.25% dermal reactions in animals could not be read due to discolouration. It was not irritating to human skin at a concentration of 8% in petrolatum, nor was it irritating to animals at concentrations of ≤ 3.125% in water. Undiluted pseudoionone was slightly irritating in an OECD 405 eye irritation test with rabbits and also in two alternative *in vitro* tests; the *in vivo* test results show transient signs of irritation but would not warrant a classification of irritating to the eyes according to the EU criteria. No respiratory irritation data have been located. Hence, pseudoionone is taken to have a strong dermal irritation potential down to concentrations below 10%, whereas the effects of pseudoionone on the eyes are slight and only transient.

3.1.4 Sensitisation

One skin sensitisation study in animals was located as well as a secondary source for human data.

Studies in Animals

Skin

Csato and Chubb [1996] reported a GLP OECD 406 maximisation test with guinea pigs. Detailed data are given regarding supplier, acclimatisation, animal husbandry, test procedures and observations with grading scores (please see SIDS for details). In the topical irritancy ranging study with 100%, 50%, 25% and 12.5% pseudoionone as a suspension in water aplied to clipped skin, pseudoionone caused both intense irritant reactions and brown staining at most test sites, preventing a full assessment of the skin responses. The incidence of obscured sites was related to the test article concentration. Based on the intradermal and topical range-finders, a main study using 20 test and 10 control animals was initiated using concentrations of 5% v/v pseudoionone in water for the intradermal induction and 50% v/v in water for the topical induction. However, following the topical induction phase the behaviour and general condition of the animals indicated that a severe skin response had occurred. The first study was therefore aborted and the main study resumed using untreated animals and a lower concentration for the topical induction phase.

In the definitive main study with, first, an intradermal induction, in 20 test animals and 10 control animals, the dorsal area between the shoulders of each animal was clipped free of fur and 3 pairs of intradermal injections were made within this area. The dose volume of each injection was 0.1 ml and each pair of injections consisted of 50% v/v Freund's Complete Adjuvant (FCA) emulsified with water, 5% v/v pseudoionone in water and 5% v/v pseudoionone in 1:1 FCA:water in the test animals and 50% v/v FCA emulsified with water, 100% water and 50% v/v water in 1:1 FCA:water in the controls. Twenty-four hours after administration of the intradermal injections, all animals were examined for signs of irritation in the treated area.

For the second, topical induction, 6 days after intradermal induction, the area surrounding the injection sites of all test and control animals was again clipped free of fur and painted with 0.5 ml of 10% w/v sodium lauryl sulfate in light liquid paraffin. The following day, patches of Whatman No 3 filter paper, 4×2 cm, each saturated with 12.5% v/v pseudoionone in water, were placed over the injection sites of all animals in the test group in order to boost the induction process. These were covered with 'Blenderm' as an occlusive barrier and the whole assembly held in place by wrapping the trunk of each animal with a length of 'Elastoplast'. Animals of the control group were similarly treated, the patch of filter paper being saturated with water. The patches and dressings were removed after 48 hours. A further 24 hours after removal of the patches all animals were re-examined for signs of irritation in the treated area.

For the topical challenges in the main study, 14 days after the topical induction application, the fur was clipped free of fur and 2×2-cm patches of filter paper, each saturated with 6.25% v/v pseudoionone in water, were placed on the left flank of all test and control animals. The right flank of each test and control animal was similarly treated with a patch soaked with water alone. The patches were occluded and secured using the method described above. At 24 hours after challenge patch removal, the sites on 19 test animals an 6 controls treated with 6.25% v/v pseudoionone in water could not be assessed for reaction to treatment because of intense red-brown skin staining. At 48 hours after the end of the occlusion period, the treated sites on 14 test and 5 control animals could still not be assessed.

Therefore, a topical re-challenge was conducted 7 days later under the same conditions as the initial challenge, using pseudoionone concentrations of 3.125% and 1.563% in water. Again, reactions were scored at 24 and 48 hours after removal of dressings and patches. Slight brown staining was apparent on the treated sites on most animals, but this did not prevent the assessment of skin reactions.

None of the animals in the test or control groups responded positively to either test article concentration at 24 or 48 hours of observation. However, it was irritating to the skin at all topical concentrations of 10% and higher, while reactions at 6.25% could not be assessed due to staining of the skin. Csato and Chubb [1996] concluded that the reactions observed during the range-finding and main studies were solely due to the irritancy of pseudoionone but not to sensitising potential. Based on this guinea pig maximisation test according to OECD 406 under GLP, pseudoionone is not a dermal sensitiser.

Studies in Humans

Skin

As reported by Ford and colleagues [1988], four maximisation test series with pseudoionone were carried out on a total of 108 volunteers by Kligman [1976, unpublished] and Epstein [1978, unpublished] on behalf of RIFM. The substance was tested at a concentration of 8% in petrolatum, this concentration was chosen "based on a reported maximum concentration of 0.8% in consumer products". As a result, "2/25 (Kligman, 1976), 4/25 (Epstein, 1978), 2/25 (Kligman, 1976) and 1/33 (Epstein, 1978) sensitization reactions" were produced, without further details as to the reactions. This corresponds to a total incidence of 9 positives out of 108 subjects or 8.3%. No further details can be derived from the publication and the original reports were never published, hence there is no information on possible earlier exposure of the probands to pseudoionone or similar substances. On the other hand, the original reports are from highly experienced and respected dermal toxicologists, therefore they are accepted as dependable in spite of lacking documentation.

Based on the report by Ford and co-workers [1988], both the International Fragrance Association [IFRA, 1979] and subsequently the European Union Scientific Committee on Cosmetic Products and Non-Food Products [SCCNFP, 2000a, 2000b] recommended a ban of the use of pseudoionone as a fragrance ingredient but tolerated it as an impurity of ≤ 2% in various ionones.

From the Teranol Lalden productioon plant, no toxic effects hinting at sensitisation have been reported during many years of occupational handling [Hauser, 2002].

Conclusion

Based on a guinea pig maximisation test according to OECD 406 under GLP [Csato & Chubb, 1996], pseudoionone is not a dermal sensitiser. All reactions to topical concentrations of 10% and higher (and to intradermal inductions at much lower concentrations) were ascribed to the irritating potential of pseudoionone, while reactions at 6.25% could not be assessed due to staining of the skin. In the four test series with human volunteers summarily reported by Ford and colleagues [1988], pseudoionone at a concentration of 8% in petrolatum produced otherwise undescribed "sensitization reactions" in 9 out of 108 subjects. Lacking further details as to the tests with human probands, pseudoionone must be accepted to have sensitising potential. Even though the conclusion from the animal test suggests an irritant rather than a genuinely sensitising mechanism of action, in view of the human data it is well possible that the described colouration contributed towards false negative results.

3.1.5 Repeated Dose Toxicity

Repeated dose oral toxicity data for pseudoionone have been located in a 28-day test, in a one-generation reproductive toxicity study and in two older 5-day tests.

Table 6 Repeated dose oral (gavage) toxicity data

Test	Result	Reference/comment
28-day oral toxicity test, OECD 407, rat, GLP	**NOAEL 250 mg/kg bw/d NOEL 50 mg/kg bw/d**	**dose-groups of 0 (vehicle, maize oil), 50, 250 and 1000 mg/kg bw/d, 28 d treatment, 14 d treatment-free period for groups 0 and 1000 mg/kg bw/d to follow reversibility; Strobel & Lambert, 1997**
one-generation reproductive toxicity test, OECD 415, rat, GLP	NOAEL 120 mg/kg bw/d NOEL 40 mg/kg bw/d	dose-groups of 0 (vehicle, maize oil), 40, 120 and 360 mg/kg bw/d, mean treatment period in males 106 (range 104–108) d, in females 60 (36–65) d; Beekhuizen, 2003
5-day repeated oral toxicity test, rat	LD_{50} = 2880 mg/kg bw/d LD_0 = 2000 mg/kg bw/d	dose-groups of 500, 1000, 2000, 4000 and 8000 mg/kg bw/d; 5 daily administrations, then 10 days observation; Bächtold, 1975
5-day repeated oral toxicity test, mouse	LD_{50} = 4550 mg/kg bw/d LD_0 = 2000 mg/kg bw/d	dose-groups of 1000, 2000, 4000 and 8000 mg/kg bw/d; 5 daily administrations, then 10 days observation; Bächtold, 1973

Studies in Animals

Oral

Strobel and Lambert [1997] performed a 28-day repeated dose oral (gavage) toxicity test according to OECD 407 under GLP with rats. 36 male and 36 female healthy acclimatised HsdBrl:WH (Wistar Hannover) strain rats were randomly assigned to four test groups: 6 males and 6 females to 50 mg/kg bw/d; 6 m & 6 f to 250 mg/kg bw/d; 12 m & 12 f to 1000 mg/kg bw/d; 12 m & 12 f to 0 mg/kg bw/d (maize/corn oil only, vehicle controls). The last 6 animals of each sex in groups 1000 and 0 (controls) mg/kg bw/d were tagged to be maintained for an additional 14-day treatment-free period in order to follow reversibility of effects. Full details as to supplier, acclimatisation and animal husbandry are given. Pseudoionone was formulated in maize/corn oil, with separate formulations prepared daily for each dose level. Concentrations were analytically confirmed and included in the full test report. A constant volume of 5 ml/kg bw/d was used, individual doses were adjusted according to the most recent body weight recorded.

All animals were examined twice daily for mortality and morbidity. All visible signs of reactions to treatment were recorded daily. All animals were weighed at the start of the study and then twice weekly up to and including the day of killing and necropsy. Blood and urine samples were obtained from the first 6 males and females in each group during week 4 of treatment. Further blood and urine samples were obtained from the remaining animals towards the end of the treatment-free period. Haematological examinations included morphological, volume, coagulation and blood chemistry parameters, similarly for urinalyis; full details are given in the report. At the end of the treatment and treatment-free periods, the designated animals were killed; before necropsy, each animal was weighed and examined externally. Any abnormalities observed during macroscopic examination were recorded. The following organs were weighed: adrenals, brain, epididymides, heart, kidneys, liver, ovaries, spleen, testes and thymus. Samples for histology were taken from 39 different organs or parts thereof (full list in report) as well as from all gross lesions. All tissues from all control and 1000-mg/kg-bw/d animals, including those allocated to the treatment-free period, plus all gross lesion samples from all animals, were examined microscopically.

The observations analysed were bodyweight at start, bodyweight gains over the regular dosing duration and over the treatment-free period, food consumption over the same intervals and absolute as well as body-weight-related organ weights. Clinical pathology data were also analysed. The

sexes were analysed separately. The data were subjected to analysis of variance, with further tests to assess potential group differences and pairwise comparison of all treatment groups with controls. The following results were analysed non-parametrically: after week 4 haematological, biochemical and urinalytical data, after week 6 biochemistry, but no haematology nor urinalysis data (full details in SIDS).

There were no mortalities in this study. Salivation was recorded on a number of occasions pre-dosing in some animals of the 250 and 1000 groups and post-dosing in all animals of the 250 and 1000 groups. These findings were considered to be treatment-related. Several other clinical signs were recorded, none of which was considered to be related to treatment. Males (but not females) of the 1000 group showed a marked, significant reduction in bodyweight gain at the end of the treatment period (-25%), however, during the treatment-free period, the bodyweight gains of males from the 1000 and 0 groups were comparable. There was no apparent effect on the bodyweights of females during treatment, however, over the treatment-free period the females from the 1000 group gained less weight than their comparable controls; this finding was considered fortuitous. There were no treatment-related effects on food consumption.

There was a slight increase in the group mean red blood cell (+6.6%) and packed cell volume values (+7.2%) for females of the 1000 group. Increased group mean platelet values were also noted in males of the 1000 group, compared to the control group mean, which was considered to reflect the low value for one control animal rather than any response to treatment. There was a small but significant increase in activated partial thromboplastin time in males of the 250 and 1000 groups (+24.7% and +28.0%). At the end of the treatment-free period, a light but statistically significant increase in packed cell volume was still observable in females of the 1000 group (+7.2%). Remaining differences in mean activated partial thromboplastin value for males of the 1000 group and mean red blood cell value for females of the 1000 group to their respective control groups after the treatment-free period did not achieve statistical significance and these parameters were considered to have recovered.

Small, statistically significant increases in alanine aminotransferase were observed in males from the 250 and 1000 groups (+36.7% and +53.3%) and in females from the 1000 group (+65%). An increase in gamma-glutamyl transpeptidase in both sexes was observed in the 1000 group (3 U/l compared to 0 U/l control value). Slight increases in total protein (+9.2%), globulin (+14.3%) and cholesterol levels (+36.8%) were observed in females from the 1000 group. Triglycerides were reduced in males of the 1000 group (–57.3%). Other, minor changes were observed which were within quoted background ranges and therefore not consideredrelated to treatment. At the end of the treatment-free period in the 1000 group no findings were recorded that were considered of toxicological significance. After 4 weeks of treatment, minor changes in urobilinogen, volume and protein levels were observed but these were considered to be coincidental and not related to treatment.

Absolute and bodyweight-related liver and kidney weights were increased in males (bodyweight-related +37.5% and +35.9%, respectively) and females of the 1000 group (bodyweight-related +50.9% and +8.5%, respectively). Increased relative kidney weights were also seen in males of the 250 group (+14.5%). A number of other, statistically significant relative organ weight changes were observed in males of the 1000 group, however, these were considered to be due to the reduced overall bodyweight and not directly related to treatment with the test article. At the end of the treatment-free period there were no significant differences from controls for liver or kidney weight in both sexes. There were no other changes considered to be of toxicological significance. No treatment-related abnormalities were observed during necropsy and histopathology. A small number of histological findings were within the normal range of background alterations seen in untreated rats of this age and strain.

In summary, daily oral administration by gavage of pseudoionone to HsdBrl:WH rats for 28 days at a dose of 1000 mg/kg bw/d was associated with the following findings: intermittent pre- and post-dose salivation, reduction in bodyweight gain in males, an increase in liver and kidney weights and a few minor changes in haematology and blood chemistry. Administration of 250 mg/kg bw/d was associated with post-dose salivation on a number of occasions and a slight increase in relative kidney weight in males. Administration of 50 mg/kg bw/d did not result in any toxicological findings. In the 1000-mg/kg-bw/d group, there were no histopathological correlates of the increased liver and kidney weights and there were no residual observations at the end of the 2-week treatment-free period, which shows that even the effects noted at 1000 mg/kg bw/d were of a transitory nature. In this study, a conservative NOAEL of 50 mg/kg bw/d based on minor reversible effects (salivation, kidney and liver weight gains) could be determined.

Additional, even longer-term repeated dose oral toxicity data resulted from a GLP one-generation reproductive toxicity study according to OECD 415 [Beekhuizen, 2003]. In brief, 96 male and 96 female Wistar rats Crl: (WI) BR (outbred, SPF quality) were exposed by daily gavage to pseudoionone in maize/corn oil as the vehicle at a dose volume of 5 ml/kg bw/d. Dose levels were 0 (vehicle controls), 40, 120 and 360 mg/kg bw/d for the four groups; these dose levels were based on the above 28-day subchronic toxicity study. The males were exposed for 11 weeks prior to mating up to termination; the mean exposure was 106 days, with a range from 104 to 108 days. The females were exposed for 2 weeks prior to mating up to termination; the mean duration of treatment was 60 days, with a range of 36 to 65 days. Full details as to supplier, acclimatisation, animal husbandry, test procedures, observations and conclusions are given in chapter 3.1.8, Toxicity to Reproduction and in the respective SIDS chapter 5.8.

There were 3 unscheduled deaths (including 2 killed *in extremis*) out of the 192 parental animals; all 3 animals were females that were found to have severe delivery difficulties. These deaths were considered incidental, probably caused by the big litter sizes, and therefore were considered not to be treatment-related. Salivation was observed in all males and females of the highest dose group. Incidental findings consisted of alopecia, lethargy, clonic spasms, rales, salivation, scabs, nodule at the tail, red staining of the right eye, broken teeth, hunched posture, piloerection, pale appearance, emaciation, dull eyes and dark eyes. Either no relationship was established with treatment for these observations or they were considered to be within the normal biological variation for rats of this age and strain. Body weights and body weight gain rates were unaffected by treatment up to 360 mg/kg bw/d. Statistically significant increases in relative food consumption were observed in some of the 120 and 360 mg/kg bw/d males. No explanation for this increase can be given, however, this finding was not considered an adverse effect, it was considered incidental in nature and not to be toxicologically relevant.

On macroscopic examination, no treatment-related findings were identified but a number of findings that were considered incidental in nature, including pelvic dilation of the left, right or both kidneys, testes reduced in size, flaccid testes, enlarged testes, accentuated lobular pattern of the liver, pale discolouration of the liver, alopecia at several parts of the body, dark red discolouration of the mediastinal cranial lymph nodes, isolated yellowish hard nodule at the tail of the left epididymis, dark red hard nodule at the left and right tips of the epididymides, epididymides reduced in size, enlarged liver, reddish soft nodule at the papillary process of the liver, soft nodule at the papillary process of the liver, stomach and spleen grown together with a soft nodule at the papillary process of the liver, dark red discolouration of the left mandibular lymph node. These findings are occasionally seen among rats used in this type of study and, in the absence of correlated microscopic histopathological findings, were not considered of toxicological significance. Fluid in the uterus (in one female of the control group, in three of the 40 mg/kg bw/d group, in one of the 120 mg/kg bw/d group and in one of the 360 mg/kg bw/d group) is related to a stage in the oestrous cycle and is a normal finding. Males and females of the 360 mg/kg bw/d group showed statistically significant

increased absolute and relative liver and kidneys weight. Males of the 120 mg/kg bw/d group showed significantly increased liver weight. In the absence of histopathological changes, both effects were considered not to be toxicologically relevant but rather manifestations of physiological adaptation to additional metabolic and excretionary loads. Males of the 40 mg/kg bw/d group showed significantly reduced seminal vesicles weight. In the absence of a dose-response relationship, this finding was considered to be caused by chance and not to be related to treatment.

On microscopic examination, there were no treatment-related findings. No histopathological changes were found to correlate with the observed increase in liver and kidney weights.

In summary, no effects that were regarded as adverse were seen at 120 mg/kg bw/d during an average exposure of 106 (range 104–108) days in males respectively 60 (36–65) days in females. In the absence of histopathological changes, even those effects noted at 360 mg/kg bw/d (increased liver and kidney weights) can be related to the additional metabolic and excretionary load and are not necessarily adverse in nature. However, including also salivation that was observed in all animals of the 360 mg/kg bw/d group, the parental toxicity NOAEL in this study, which was of subchronic duration for the females and of chronic duration for the males was 120 mg/kg bw/d and the NOEL was 40 mg/kg bw/d.

Further repeated dose oral toxicity data were reported by Bächtold [1973, 1975] for mice and rats. In former Roche standard short-term tests, groups of 10 mice or rats from the in-house inbred strains were dosed by gavage once daily for 5 days and observed for a further 10 days, when the test was terminated by killing and dissecting all survivors. Controls were historical with the same strains. Statistics were computed if applicable. Dr Bächtold produced a lot of acute and subchronic toxicological data, but in line with the time (1960s to mid-1980s) and in view of the envisaged internal use for the results, the reports are very brief. The results are still considered reliable due to the combination of a professional toxicology lab run by the same personnel for many years with highly standardised test procedures, testing many compounds and a relatively high number of animals dosed per concentration, which makes for good quality and dependable interpolated results.

In the test with mice [Bächtold, 1973], 4 groups of 10 mice each were dosed with either 1000, 2000, 4000 or 8000 mg pseudoionone/kg bw/d for 5 consecutive days. On day 1 of administration, 8 mice of the 8000 group were dead; on day 2, all 10 mice in the 8000 group were dead; on day 5, 1 mouse of the 4000 group was dead; finally, on day 15, 10 days after the 5th and last dose, still 1 mouse of the 4000 group was dead; all mice from all lower-dose groups survived. A slight reduction in body weight gain of the survivors was seen in the 4000 and 2000 groups between day 0 (the day before the first administration) and day 6. At the end of this study, the LD_{50} for mice was 4550±640 mg/kg bw/d and the LD_0 was 2000 mg/kg bw/d.

In the test with rats [Bächtold, 1975], 5 groups of 10 rats each were dosed with either 500, 1000, 2000, 4000 or 8000 mg pseudoionone/kg bw/d for 5 consecutive days. On day 3 of administration, 7 rats of the 8000 group and 1 rat of the 4000 group were dead; on day 4, all 10 rats in the 8000 group and 2 rats of the 4000 group were dead; on day 5, 5 rats of the 4000 group were dead; finally, on day 15, 10 days after the 5th and last dose, 6 rats of the 4000 group were dead; all rats from all lower-dose groups survived. General symptoms of the rats are described as “sedation” in the 8000, 4000 and 2000 groups and as “light sedation” in the 1000 and 500 groups. A slight reduction in body weight gain of the survivors was seen in the 4000 and 2000 groups between day 0 (the day before the first administration) and day 6. At the end of this study, the LD_{50} for rats was 3880±620 mg/kg bw/d and the LD_0 was 2000 mg/kg bw/d.

Conclusion

Results from available repeated dose oral toxicity studies by gavage give a consistent picture. In the 28-day OECD 407 GLP test with rats [Strobel & Lambert, 1997], the NOAEL was 50 mg/kg bw/d.

But even the effects noted at the highest dose of 1000 mg/kg bw/d, *viz.* increase in liver and kidney weights in both sexes and reduced bodyweight gain in males, had no histopathological correlates and were of a transitory nature as evidenced by their resolution at the end of the 2-week treatment-free period.

Data from an OECD 415 one-generation GLP test with rats [Beekhuizen, 2003] show the same picture. With much longer average exposures of 106 (range 104–108) days in males and 60 (36–65) days in females to 40, 120 or 360 mg/kg bw/d, no effects that were regarded as adverse were seen at 120 mg/kg bw/d. In the absence of histopathological changes, even those effects noted at the highest dose of 360 mg/kg bw/d, increased liver and kidney weights, can be related to the additional metabolic and excretionary load and are not necessarily adverse in nature. The parental toxicity NOAEL was 120 mg/kg bw/d and the NOEL was 40 mg/kg bw/d, very similar to the above study.

Two shorter-term older studies with rats and mice [Bächtold, 1973, 1975] with daily gavage for 5 days and 10 days' additional observation showed sedation and reduced body weight gain over the treatment period in the survivors, an LC_{50} of 3880 and 4550 mg/kg bw/d for rats and mice, respectively, and a nonlethal daily dose of 2000 mg/kg bw/d for both.

Based on two subchronic studies under GLP, pseudoionone has a low repeated dose toxicity based on minor reversible effects that however were consitently observed in both sudies. These effects included kidney and liver weight changes as well as salivation with NOAELs of 50 and 120 mg/kg bw/d and also the highest tested doses of 360 and 1000 mg/kg bw/d resulted in low-level effects that may be explained as physiological adaptation and which resolved during the treatment-free period of the 28-day study. Additional shorter-term data over 5 days of administration with much higher doses confirm the low toxicity.

3.1.6 Mutagenicity

Two bacterial *in vitro* and one mammalian *in vivo* mutagenicity studies have been located for pseudoionone.

Table 7 Mutagenicity data

Test	Result	Reference/comment
Micronucleus assay, OECD 474, GLP	no increase in micro-nucleated polychromatic erythrocytes	mouse, gavage, 2000 mg/kg bw; Buskens, 2003
Ames test, OECD 471, GLP, *Salmonella typhimurium*	not mutagenic	TA1535, TA97, TA98, TA1000, TA102, with and without S9 metabolic activation, with liquid pre-incubation; strain-dependent toxicity; Albertini, 1996
Ames test, *S. typhimurium*	not mutagenic	TA98, TA100, TA1535, TA1537, with and without S9 metabolic activation; Florin *et al.,* 1980

In vivo Studies

A GLP micronucleus test was conducted by Buskens [2003] with NMRI BR mice, pseudoionone as the test substance and cyclophosphamide as a positive control. Full details as to supplier, acclimatisation, animal husbandry, test procedures and statistics are given. Three males and 3 females were used for the dose range-finding test at 2000 mg/kg bw by gavage. As all animals survived the range-

finder and as there were no obvious differences between sexes, 5 males each were used per test group respectively as negative and positive controls, distributed in 4 groups labelled A through D. A was a negative control (vehicle only, 10 ml maize/corn oil/kg bw), groups B and C were treatment groups (2000 mg pseudoionone/kg bw in maize/corn oil, dose adjusted to a volume of 10 ml/kg bw; group B to be sampled at 24 h post-dosing, group C at 48 h post-dosing) and D was a positive control group (50 mg cyclophosphamide/kg bw in physiological saline). The test animals were killed by cervical dislocation 24 h (groups A and B) respectively 48 h (groups C and D) after dosing. In every instance, marrow cells from both femurs were removed, prepared for microscopical examination and scored at ×1000. The number of micronucleated polychromatic erythrocytes was counted in a total of 2000 polychromatic erythrocytes per slide. The ratio of polychromatic to normochromatic erythrocytes was determined in the first 1000 erythrocytes scanned. Micronuclei were only counted in polychromatic erythrocytes. A two-sided test Wilcoxon Rank Sum test was applied to detect statistically significant increases in the frequency of micronucleated polychromatic erythrocytes, at any dose or sampling time, would be using.

All animals treated with 2000 mg/kg bw and both the negative and positive controls showed no abnormalities. The average numbers (N) of micronucleated polychromatic erythrocytes per 2000 polychromatic erythrocytes and ratios (R) of polychromatic to normochromatic erythrocytes were as follows: Group A, vehicle controls, 24 h, N = 2.2±1.5, R = 1.16±0.13; B, 2000 mg pseudoionone/kg bw, 24 h, N = 1.4±1.1, R = 1.20±0.10; C, 2000 mg pseudoionone/kg bw, 48 h, N = 1.8±1.5, R = 1.07±0.06; D, cyclophosphamide 50 mg/kg bw, 24 h, N = 44.4±10.6 ($p < 0.01$), R = 0.29±0.07. Hence, at an oral dose of 2000 mg/kg, bw pseudoionone did not induce any increase in the incidence of micronucleated polychromatic erythrocytes in this in vivo mouse test and is therefore regarded as negative regarding genotoxic effects in this model. Further, the test groups treated with pseudoionone did not show any decrease in the ratio of normochromatic to polychromatic erythrocytes, which reflects a lack of toxic effects of pseudoionone on erythropoiesis.

In vitro Studies

Albertini [1996] reported the results of a GLP Ames test according to OECD 471, with *Salmonella typhimurium* strains TA1535, TA97, TA98, TA1000, TA102, with and without S9 metabolic activation from phenobarbital/beta-naphthoflavone-treated rats, with and without liquid pre-incubation. A toxicity prescreen with TA100 and solvent controls showed toxic effects (reduced background growth, reduction in the number of revertant colonies) starting at 500 µg/plate, which was chosen as the highest test concentration. For the standard Ames procedure, pseudoionone concentrations were 50, 166 and 500 µg/plate. Three replicate plates for every test compound concentration with and without S9 mix and the negative control, plus two replicate plates for every positive control (sodium azide; ICR191; 2-nitrofluorene; MMC) were incubated upside down at 37 °C for 2 days. In the liquid pre-incubation assay, pseudoionone, solvent only or positive substances (see above), S9 mix where scheduled and overnight culture broth for the respective strain were mixed and incubated on a shaker for 30 minutes at 37 °C. Then soft agar supplemented with histidine/biotin was added, the tubes mixed and the contents immediately poured onto Vogel-Bronner minimal agar plates and incubated upside down at 37 °C for 2 days. Colonies were counted electronically, the background lawn was inspected using a microscope for toxicity; absence or presence of a confluent bacterial lawn was recorded and interpreted as toxicity or non-toxicity fo the test substance.

Strain-dependent toxicity was noted with both methods used. In the liquid preincubation assay, toxicity was noted already at 50 µg/plate for strains TA1535 and TA102in the absence of S9 mix. Therefore a repeat experiment using the preincubation method was performed in the concentration range of 0.5-50 µg/plate with these strains, with and without S9 mix. Neither in the standard Ames plate incorporation assay nor in the liquid preincubation assay were increases in mutant colony frequency noted with any of the 5 strains, with or without S9 mix. The mutant frequencies of the controls were in the historical range of controls. In summary, neither pseudoionone per se nor any

of its S9-mix metabolites were mutagenic in standard Ames and liquid preincubation bacterial mutagenicity assays with five different strains of *S. typhimurium*.

In an older publication, Florin and co-workers [1980] screened various tobacco smoke compounds including pseudoionone for mutagenicity in assay according to Ames and colleagues [Mutat Res *31:* 347–364, 1975] with histidine-requiring *S. typhimurium* strains TA98, TA100, TA 1535 and TA1537. Revertants were scored on glucose minimal salts medium supplemented with 0.05 µmol histidine and 0.05 µmol biotin. 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. S9 fractions for metabolic activation were prepared as described by Ames and co-workers (see above). Aroclor 1254 or 3-methylcholanthrene, both suspended in maize/corn oil, were used as inducers in male Sprague-Dawley rats. Full details as to the test procedure and the preparation of the S9 mix are in the publication. Most test compounds, including pseudoionone, were dissolved in ethanol for incorporation into the plates. Pseudoionone was not mutagenic to strains TA98, TA100, TA1535 or TA1537 in an Ames test with and without metabolic activation at a concentration of 3 µmol/plate (= 577 µg/plate). While this is an older publication that states the negative results (as for pseudoionone) only summarily, the detailed methods, positive controls and quality control measures support the credibility of the data.

Conclusion

Pseudoionone did not show any increase in micronucleated polychromatic erythrocytes in a GLP *in vivo* micronucleus assay [Buskens, 2003] nor an increased number of revertant colonies in two *in vitro* bacterial Ames tests [Albertini, 1996; Florin *et al.,* 1980], both rated reliable and more recent one also under GLP. Based on these consistent results, pseudoionone is not suspected of any mutagenic activity.

3.1.7 Carcinogenicity

No data have been located.

3.1.8 Toxicity for Reproduction

A one-generation reproductive toxicity study in rats according to OECD 415 was performed under GLP by Beekhuizen [2003]. Additional reprotox data were located in an older study by Willhite [1986] in hamsters. The methods and general results of both studies are presented below in abbreviated form, the specific results under the subheadings “Effects on fertility” and “Developmental toxicity” further down.

In the recent OECD 415 study by Beekhuizen [2003], 96 young (5- to 6-week-old) male and 96 slightly older (11- to 12-week-old) female healthy Wistar rats Crl:(WI)BR (outbred, SPF quality) were assigned by computer-generated randomisation to four test groups. Full details as to supplier, acclimatisation, animal husbandry, test procedures and statistics are given. Dose levels were 0 (vehicle controls), 40, 120 and 360 mg pseudoionone/kg bw/d for the four groups, formulated daily using maize/corn oil; these dose levels were based on the above GLP 28-day subchronic toxicity study. Dosing was by gavage at a volume of 5 ml/kg bw, actual volumes were calculated according to the latest individual body weights. The males were exposed for 11 weeks prior to mating up to

termination; the mean exposure was 106 days, with a range from 104 to 108 days. The females were exposed for 2 weeks prior to mating up to termination; the mean duration of treatment was 60 days, with a range of 36 to 65 days. The offspring was not treated.

Females were paired one-to-one with males from the same group. After mating was confirmed by a copulation plug, the males and females were separated. The pregnant females were allowed to litter normally. Day 1 of lactation was defined as the day when a litter was found completed (i.e., membranes, placentas cleaned up, nest built up and/or feeding of pups started). On day 4 after birth the size of each litter was adjusted at random by eliminating extra pups to yield, as closely as possible, four male and four female pups per litter (details in SIDS). At the end of the study, all survivors were killed, the males after confirmation of the pregancy or successful delivery of the female they were mated with, the females at day 21 post partum or shortly thereafter. Pups were killed either at adjusting litters on day 4 post partum or at the end of the study at day 21 post partum.

Parental animals were observed twice daily for behavioural and clinical signs, the latter were recorded according to fixed scales. Cage debris of pregnant females were examined to detect abortion or premature birth. Signs of difficult or prolonged parturition were recorded. Males and females were weighed on the first day of exposure and weekly thereafter. Mated females were weighed on days 0, 7, 14 and 21 of gestation and during lactation on days 1, 4, 7, 14 and 21. Food consumption was recorded weekly for males and females, with exception of the mating period. Food consumption of mated females was recorded on gestation days 0, 7, 14 and 21 and during lactation on days 1, 4, 7, 14 and 21. Regarding water consumption, subjective appraisal was maintained during the study as there were no suspicions of any effect of treatment. Reproductive basic data such as numbers of animals mated, mating date, confirmation of pregnancy and day of delivery were recorded. For the offspring, the numbers of live and dead pups at first litter check (= day 1 of lactation) and daily thereafter was recorded as well as the individual weight of all live pups on days 1, 4, 7, 14 and 21 of lactation, the sex of the pups by assessment of the ano-genital distance, the number of pups with physical or behavioural abnormalities.

After killing or natural death all parental animals were subjected to external examination and to macroscopic examination during dissection, with special attention to the reproductive organs. The terminal body weight and the following organ weights were recorded: cervix plus uterus, epididymides, kidney, liver, ovaries, pituitary, prostate, seminal vesicles together with coagulating gland and fluids, spleen and testes. During dissection, samples of the following organs and tissues were collected and fixed for histopathology: all gross lesions, cervix, coagulation gland, epididymides, kidneys, liver, ovaries, pituitary, prostate, seminal vesicles, spleen, testes, uterus and vagina. In case a female was not pregnant, the whole uterus was stained after Salewski in order to determine any early post-implantation losses through evidencing implantation site scars. Microspcopic slides were prepared and examined by a professional histopathologist, abnormalities were described and included in his report.

Pups were sexed, externally examined and subjected to external examination of the thoracic and abdominal tissues and organs, their stomach examined for the presence of milk. All abnormalities were recorded and, if possible, defects or cause of death were evaluated.

Thirteen protocol deviations are listed, which were evaluated and considered not to have affected the integrity of the study or of the results. There were 3 unscheduled deaths out of a total of 192 main parental animals; all 3 animals were females. Two were killed *in extremis,* one each in the 120 and the 360 mg/kg bw/d groups after 38 respectively 43 days of treatment. The other animal, also a female from the 360 mg/kg bw/d group, died spontaneously on day 38. All three were found to have severe delivery difficulties, with 17 foetuses in the birth canal, 16 dead pups and three foetal resorptions, and 19 foetuses in the birth canal, respectively. These deaths were considered incidental, very possibly caused by the big litter sizes and not to be related to the treatment with the test substance.

Salivation was observed in all males and females of the highest dose group. Incidental findings consisted of alopecia, lethargy, clonic spasms, rales, salivation, scabs, nodule at the tail, red staining of the right eye, broken teeth, hunched posture, piloerection, pale appearance, emaciation, dull eyes and dark eyes. No relationship was established with treatment for these observations or they were considered to be within the normal biological variation for rats of this age and strain. Animal no. 40 of the 40 mg/kg bw/d group showed transient signs of stress (compulsive biting, saltator spasms, tremor and muscle twitching) just before or after dosing during four days of treatment.

Body weights and body weight gain rates were unaffected by treatment up to 360 mg/kg bw/d. Significant increases in relative food consumption were observed in some of the 120 and 360 mg/kg bw/d males. This finding was considered incidental in nature, not an adverse effect and not toxicologically relevant.

No treatment-related macroscopic findings were identified but a number of findings that were considered incidental in nature, ncluding pelvic dilation of the left, right or both kidneys, testes reduced in size, flaccid testes, enlarged testes, accentuated lobular pattern of the liver, pale discolouration of the liver, alopecia at several parts of the body, dark red discolouration of the mediastinal cranial lymph nodes, isolated yellowish hard nodule at the tail of the left epididymis, dark red hard nodule at the left and right tips of the epididymides, epididymides reduced in size, enlarged liver, reddish soft nodule at the papillary process of the liver, soft nodule at the papillary process of the liver, stomach and spleen grown together with a soft nodule at the papillary process of the liver, dark red discolouration of the left mandibular lymph node. Such findings are occasionally seen among rats used in this type of study and, in the absence of correlated microscopic histopathological findings, were not considered of toxicological significance. Fluid in the uterus (in one female of the control group, in three of the 40 mg/kg bw/d group, in one of the 120 mg/kg bw/d group and of the 360 mg/kg bw/d group) is related to a stage in the oestrous cycle and is a normal finding. In the 120 mg/kg bw/d group, one female that was killed *in extremis* showed 17 foetuses in the birth canal. Of the 360 mg/kg bw/d group, one female that was killed *in extremis* showed 3 foetal resorptions and 9 placentas in the left uterus horn and the thoracic cavity containing milky-cloudy fluid; one female from the 360 mg/kg bw/d group that died spontaneously showed 19 foetuses in the birth canal and beginning autolysis.

Males and females of the 360 mg/kg bw/d group showed statistically significant increased absolute and relative liver and kidneys weight (relative liver weight males +23.1%, females +24.0%; relative kidney weight males +11.6%, females +13.2%). Males of the 120 mg/kg bw/d group showed significantly increased relative liver weight (+7.4%). In the absence of histopathological changes, both effects were not considered toxicologically relevant but as physiological adaptation to additional metabolic and excretionary loads. Males of the 40 mg/kg bw/d group showed significantly reduced seminal vesicles weight. In the absence of a dose-response relationship, this finding was considered to be caused by chance and not to be related to treatment.

There were no treatment-related findings in microscopic examination. No histopathological changes were found to correlate with the observed increase in liver and kidney weights.

Willhite [1986] reported the effects of a single dose of several retinoids and similar test substances including pseudoionone on female timed pregnant Syrian hamsters of strain LAK:LVG(SYR) and their foetuses. In the morning of day 8 after coition, a single dose of 96 or 960 mg pseudoionone/kg bw or vehicle (Tween 20 with 5% acetone) was given by oral gavage at a dose of 0.5 ml/100 g bw. The animals were killed by carbon dioxide asphyxiation on day 14 after coition and weighed again. The pregnant uteri were excised, numbers of resorptions and dead foetuses were recorded and living foetuses were examined under a binocular microscope. All foetuses were weighed and one-third

(approximated) of each litter was fixed and subsequently sectioned sagittally for microscopic examination. Two-thirds of each litter were fixed and whole-stained with Alizarin Red S to show skeletal (mal)formation.

The maternal weight change, calculated from the day of treatment to the day of termination, and mean litter bodyweights were analysed statistically (full details in SIDS). The number of resorptions for each test substance dose dose were compared the the vehicle control value. The incidence of abnormal litters, defined as those containing one or more malformed foetuses or three or more resorbed implantation sites, was also analysed statistically. The median effective dose for induction of terata and the embryonic LD_{50} were calculated for those retinoids associated with significant teratogenic response or elevated resorption rates.

As a general result, the administration of Tween20:acetone (95:5, v/v) alone was associated with a low incidence of embryonic and foetal death and malformation in the hamster dams.

Effects on Fertility

In the OECD 415 rat study [Beekhuizen, 2003], the reproductive parameters were unaffected by treatment up to 360 mg pseudoionone/kg bw/d. In the 40 mg/kg bw/d group, one female did not mate and one female was not pregnant. In the 120 mg/kg bw/d group, one female showed delivery difficulties, and in the 360 mg/kg bw/d group, two females showed delivery difficulties, all of which were not considered related to the treatment but rather to the high number of foetuses. All other parameters, specifically mating performance, duration of gestation and fertility parameters including number of pups at birth were similar for the control and all three treatment groups. Hence, it was concluded that the reproductive respectively fertility parameters were not affected up to 360 mg/kg bw/d. Based on the liver- and kidney-weight changes in males and salivation by all high dose animals, the general toxicological parental NOAEL was 120 mg/kg bw/d (NOEL 40 mg/kg bw/d) while the reproductive NOEL was 360 mg/kg bw/d, the highest dose level tested.

Developmental Toxicity

In the OECD 415 rat study [Beekhuizen, 2003], the development of the pups was unaffected by treatment up to 360 mg/kg bw/d. The numbers of pups at birth were similar between controls and all treatment groups (40, 120 and 360 mg/kg bw/d). No teratogenic malformations are reported. Hence, the developmental NOEL was set at 360 mg/kg bw/d.

During 21 days *post partum,* with exposure still only to the dams, the mean bodyweights of the pups in the 120 and 360 mg/kg bw/d groups, were slightly but significantly reduced (90.6% respectively 95.6% of concurrent controls). As these values were within the range of historical data, it was assumed that the significance was derived from a slightly higher mean bodyweight in the concurrent controls and that this finding was not toxicologically relevant. However, postnatal deaths were significantly increased at 360 mg/kg bw/d during days 0–4 *post partum*, due to which the viability index was decreased in this group (91.0 compared to 96.6 control). On the other hand, the number of dead and living pups at first litter check, of living pups on day 4 *post partum,* of breeding losses during days 5–21 *post partum,* of living pups on day 21 *post partum* and the weaning index were similar for control and all treated groups. In consequence, the reproductive toxicity NOAEL was set at 120 mg/kg bw/d.

In the hamster study [Willhite, 1986], a single administration of pseudoionone resulted in the following main observations (full details in the SIDS). Among the 20 controls (vehicle only), 7 low-dose (96 mg pseudoionone/kg bw) and 10 high-dose (960 mg/kg bw) animals, there was no significant difference in the incidence of total litters, of abnormal litters, of implantation sites per dam, of abnormal live foetuses, of dead foetuses or of foetal bodyweights. Only the average maternal bodyweight change (10.2±6.1 g control, 10.6±6.1 g low-dose and 5.0±6.4 g high-dose) was significantly

lower in the high-dose group compared to the controls, but no single foetal or embryonic endpoint. Hence, for a single dose of pseudoionone to pregnant hamster dams the toxicity NOEL was 96 mg/kg bw for the dams and the developmental toxicity NOEL 960 mg/kg bw for the foetuses over the short study period.

Conclusion

In a recent GLP one-generation test according to OECD 415 in rats [Beekhuizen, 2003] with gavage dose levels of 0 (vehicle controls), 40, 120 and 360 mg/kg bw/da and with mean exposure durations for males of 106 (range 104–108) days and for females of 60 (36–65) days, both the parental reproductive respectively fertility parameters and the development of the pups were not affected up to the highest tested dose of 360 mg/kg bw/d. Parental reproductive effects noted, *viz.,* one female that did not mate, one female that was not pregnant and three females that showed delivery difficulties, were not considered related to the treatment. All other parameters, specifically mating performance, duration of gestation and fertility parameters, were similar for the control and all three treatment groups. Observed liver- and kidney-weight changes in males at 120 and 360 mg/kg bw/d were interpreted as minor physiological effects that reflect increased metabolic load and are not regarded as adverse toxicological events. However salivation was observed in all animals of the top dose. These effects were consistently also observed in a 28 days repeated dose toxicity study (see 3.1.5). Developmental data show comparable numbers of pups at birth for controls and all treatment groups, moreover, no teratogenic malformations are reported. Based on this study, pseudoionone had no effect on parental reproductive and foetal developmental parameters up to the highest tested dose, corresponding to a NOEL of 360 mg/kg bw/d. However, due to an increased rate of pup deaths in the highest dose group during days 0–4 *post partum,* the reproductive toxicity NOAEL was set at the middle dose of 120 mg/kg bw/d. Taken together 120 mg/kg bw/d is the parental systemic toxicity NOAEL based on salivation, kidney and liver weight gains. The developmental NOAEL is 360 mg/kg bw/d and the overall reprotoxicity NOAEL is 120 mg/kg bw/d based on an increased rate in pup deaths during days 1-4 *post partum.*

The conclusion of no adverse effects on foetuses is supported by data from a hamster study by Willhite [1986], where no effects on foetal development were observed subsequent to one single administration of 960 or 96 mg pseudoionone/kg bw to pregnant dams, while the higher dose resulted in a reduced maternal body-weight gain in the absence of foetal effects.

Based on the available data, the reproductive and developmental toxicity associated with pseudoionone is considered to be low.

3.1.9 Other relevant toxicological information

The inhibition of the cell division of cultured murine “ascites sarcoma BP8” cells by constituents of tobacco and tobacco smoke, including pseudoionone, was tested by Pilotti and colleagues [1975]. The compounds to be tested were dissolved in ethanol and/or dimethyl sulfoxide and added to the cell suspensions (initial density of 4000 cells/ml in sterile medium), which were were incubated at 37 °C for 48 hours. All test substances were run in duplicate, 8–10 controls were run per series of test compounds. The growth rates of the duplicate cultures were calculated based on the cell counter values after 48 hours and compared to the mean of the controls. The normal growth rate for the controls was a doubling approximately every 24 hours. Pseudoionone inhibited the growth rate by 100% at both 1 and 0.1 m*M* (192 and 19.2 mg/l, respectively) and by a statistically non-significant 9% at 0.01 m*M* (1.92 mg/l). Hence, significant cellular toxicity occurs at pseudoionone concentrations above 2 mg/l, while at 20 mg/l cell division is completely inhibited; concentrations below 2 mg/l did not have a significant effect.

Thelestam and colleagues [1980] tested the effect of substances including pseudoionone on the integrity of the plasma membrane of cultured human embryonic lung cells. The cells in confluent monolayers were labelled with [^{3}H]uridine to obtain a low-molecular-weight cytoplasmic marker consisting of uridine nucleotides. Then, the cultures were exposed to the test substances at a concentration of 25 m*M* for 30 minutes at 37 °C to see whether these would exert a negative influence on the cellular plasma membrane resulting in leakage of [^{3}H]uridine into the medium. Then, the medium containing leaked radioactive marker was removed, centrifuged and the radioactivity in supernatant aliquots was determined by scintillation counter. Relative leakage of radioactive marker in per cent was calculated by dividing the difference between experimental (specific test substance) and spontaneous control release by the difference between maximal (of all test substances) and spontaneous control release and multiplying with 100. The spontaneous background release of radiomarker was 3–7% of the maximal release. Pseudoionone resulted in 68% relative release, which is in the upper range of the band termed "moderate" (15–70%). Hence, at a relatively high concentration of 25 m*M* (4800 mg/l), pseudoionone had a clear permeability-enhancing effect on cultured human lung fibroblasts.

Petterson and co-workers [1982] investigated the effect of single compounds occurring in tobacco smoke on the function of ciliated tracheal epithelium cultures prepared from chicken embryos. Transversely cut tracheal rings were exposed in a Perspex testing chamber containing the medium and the test compound; pseudoionone was dissolved in ethanol. A microscope connected to a TV camera, a TV monitor and a videotape recorder was used for automated recording of ciliary activity during the whole exposure of maximally 60 minutes. The tape was later replayed to determine time to complete cessation of ciliary activity. Substance tests were performed in triplicate involving rings from different tracheal preparations. The solvents were tested as negative controls and were found to be nontoxic to cilia at the concentration used in all experiments (1.6% v/v) with a time to cessation of ciliary activity in the blank and solvent controls of > 60 minutes, i.e., longer than the time frame for testing. Time to cessation of ciliary activity in the presence of 5 m*M* (= 962 mg/l) pseudoionone was 23 minutes. With pseudoionone, precipitates were noted in the test chambers, meaning that the actual concentration in the test medium may have been lower. However, in this screening of 300 different compounds, no substance-specific quantitative analyses were performed. Hence, pseudoionone completely inhibited ciliary activity in excised embryonic chicken tracheal epithelium at a relatively high concentration of nominally 962 mg/l within 23 minutes.

In a micronucleus test with mice [Buskens, 2003; see chapter 3.1.6], the test groups treated with a single dose of 2000 mg pseudoionone/kg bw by gavage did not show any decrease in the ratio of normochromatic to polychromatic erythrocytes, which reflects a lack of toxic effects of pseudoionone on erythropoiesis.

Hase and co-workers [1976] investigated the relative binding of Retinol-Binding Protein (RBP), a blood protein specific for retinol (= vitamin A) transport, to vitamin A derivatives and selected terpenes with structural similarities to parts of retinol, including pseudoionone. RBP was purified from the urine of patients with certain diseases. For the competitive binding experiment, 0.1 ml of pseudoionone and 4.0 ml of standardised RBP solution in buffer were mixed and left to react for 30 minutes. To this mixture, 0.2 ml of a 0.35% retinol solution was added, then the mixture was gently stirred for 10 minutes and subsequently centrifuged. The aqueous layer containing the RBP fraction was analysed in a spectrophotometer, the molar ratio of retinol to RBP was derived from the relative absorbance ratio, from which the relative respectively competitive binding was calculated, full details are given in the paper. In comparison with the retinol standard, RBP pre-exposure to pseudoionone resulted in only 25% retinol binding, respectively 75% retinol-binding inhibition. Among terpenoids, competitive binding was only higher in beta-ionone and beta-ionylidene acetic acid on one hand, both of which are characterised by a closed beta-ionone ring identical to the one in retinol, and by citral which like pseudoionone has a terminal respectively subterminal carbonyl

group. Hence, RBP showed a high affinity for pseudoionone and pseudoionone is a potential inhibitor of RBP.

Conclusion

Pseudoionone possesses a certain potential for cellular toxicity, as shown in several *in vitro* or *ex vivo* studies. Cell division in murine ascites sarcoma cell cultures was effectively inhibited at concentrations between 2 and 20 mg/l medium [Pilotti *et al.,* 1975]; at a high concentration of 4800 mg/l, pseudoionone significantly interfered with the integrity of cultured human embryonic lung cell membranes, leading to leakage [Thelestam *et al.,* 1980]; a high concentration of 960 mg/l inhibited ciliary movement of excised embryonic chicken trachea cells [Petterson *et al.,* 1980].

In contrast, an *in vivo* micronucleus test [Buskens, 2003] did not show any inhibition of erythropoiesis in mice subsequent to a single oral dose of 2000 mg pseudoionone/kg bw. As the latter dose is estimated to lead to theoretical serum concentrations well in the mg/l range, where inhibition of cell division occurred in the sarcoma cell cultures, it is concluded that metabolism, possibly hepatic first-pass metabolism, in an intact organism is capable of rapidly reducing potentially cytotoxic concentrations to safe levels.

Due to its structural similarity to the alkyl moiety of retinol, pseudoionone is a potential inhibitor of Retinol-Binding Protein [Hase *et al.,* 1976].

3.2 Initial Assessment for Human Health

Pseudoionone is of low acute toxicity by oral or dermal administration, with NOELs of 2000 mg/kg bw or higher in the gavage studies and LD_{50} values consistently >5000 mg/kg bw for both oral and dermal administration. No inhalative toxicity data are available.

In contact with skin, pseudoionone is severly to moderately irritating down to dilutions of 12.5%. Due to staining, no reading of reactions could be performed at 6.25%. No irritation was produced with concentrations of ≤ 3.125% applied to the skin. However, even lower concentrations caused irritation when injected intradermally. Hence, pseudoionone is a moderate to severe skin irritant.

In a recent OECD skin sensitisation test under GLP, pseudoionone was not rated as a sensitiser, as the reactions seen were interpreted to be due to an irritating mode of action. However, older maximisation tests resulted in 9 out of 108 human probands showing sensitisation reactions to 8% pseudoionone. Therefore, pseudoionone must be seen as a skin sensitiser.

On repeated oral administration, pseudoionone showed consistently low toxicity. In a 28-day OECD study under GLP, the NOAEL was 50 mg/kg bw/d and even the effects noted at 1000 mg/kg bw/d particularly in males, enlarged livers and kidneys without histopathological correlates, were transitory as shown by their complete regression after an additional 2-week treatment-free period. Even longer exposure, for males on the average 106 (range 104–108) days and for females of 60 (35–65) days, during an OECD one-generation reproductive toxicity study under GLP showed comparable results with a paternal toxicological NOEL of 40 mg/kg bw/d, a NOAEL of 120 mg/kg bw/d and minor effects, again enlarged livers and kidneys without histopathological correlates, at 360 mg/kg bw/d. However in both studies salivation could consistently be observed.

Pseudoionone was consistently negative in two *in vitro* bacterial Ames tests and one *in vivo* OECD micronucleus test under GLP. Thus, there is no suspicion of mutagenicity.

Pseudoionone has a low reproductive toxicity based on an OECD one-generation test under GLP. The parental systemic toxicity was set at 120 mg/kg bw/d, the the development of the pups were not affected up to the highest tested dose, corresponding to a NOAEL of 360 mg/kg bw/d. Due to an

increase in pup deaths directly after birth in the highest dose group, the overall reprotoxicity NOAEL was set at 120 mg/kg bw/d.

Based on several nonstandard *in vitro* studies, pseudoionone has a certain cytotoxic potential, in particluar at higher concentrations. In intact animals, as shown in the *in vivo* micronucleus assay, cytotoxicity seems to be prevented by rapid metabolism.

In conclusion, pseudoionone is a substance with low acute, chronic and reproductive toxicity to mammals and without mutagenic potential. However, it is a moderate to severe skin irritant and also a skin sensitiser.

4 HAZARDS TO THE ENVIRONMENT

4.1 Aquatic Effects

Acute Toxicity Test Results

Experimental data for aquatic toxicity of pseudoionone are available for fish, daphnids, green algae and bluegreen algae or Cyanobacteria. The available results are based on nominal concentrations, however, these are considered as trustworthy because a) the effect concentrations found are lower than one-tenth of the water solubility and b) pseudoionone is considered stable in the short term as there are no hydrolysable bonds on one hand and as the ready biodegradation study showed an initial lag phase of 7 days. Some further marine ecotoxicological information is hard to assess. Additionally, QSAR toxicity values for fish, daphnids and algae were calculated.

Further, analogous data for beta-ionone is presented for several individual endpoints to support data for pseudoionone. beta-Ionone has the same molecular formula as pseudoionone; its structure differs only in the closed ring in place of an isolated double bond. This results in a close correlation of some physico-chemical properties, e.g., boiling, point, vapour pressure, $\log P_{OW}$, surface tension, etc. Based on mechanistic reasoning this suggests similar toxicological and ecotoxicological properties.

Table 8 Aquatic toxicity data

Test	Result	Reference/comment
Acute toxicity to fish, *Leuciscus idus,* 96 h, DIN 38412 part 15	LC_{50} 6.8 mg/l CC NOEC 4.64 mg/l NC LC_{100} 10.0 mg/l NC	NC = nominal respectively loading concentrations, CC = calculated concentration; pH ~ 8.0, > 60% O_2 saturation, T = 20–21 °C; Kirsch & Munk, 1989
Acute toxicity to fish, *Oncorhynchus mykiss,* 48 h, corresponding to OECD 203, *test substance beta-ionone*	LC_{50} 7.1 mg/l CC NOEC 5.0 mg/l NC LC_{100} 10.0 mg/l NC	NC = nominal respectively loading concentrations, CC = calculated concentration; Gröner, 1989
QSAR baseline fish LC_{50}, TGD	LC_{50} 3.12 mg/l CC	non-polar model, based on molecular weight and $logP_{OW}$, CC = calculated concentration; TGD, Commission of the European Communities, 1996
Acute toxicity to daphnids, *Daphnia magna,* 48 h, DIN 38412 part 11	EC_{50} 3.7 mg/l CC EC_0 1.0 mg/l CC NOEC 0.58 mg/l NC EC_{100} 10.0 mg/l NC	NC = nominal concentrations, CC = calculated concentration, test performed using Cremophor emulsifier; Noack, 1990
QSAR baseline daphnid EC_{50}, TGD	EC_{50} 1.46 mg/l CC	non-polar model, based on molecular weight and $logP_{OW}$, CC = calculated concentration; TGD, Commission of the European Communities, 1996
Acute toxicity to algae, *Scenedesmus subspicatus,* 72 h, DIN 38412 part 9	E_bC_{50} 1.11 mg/l CC E_rC_{50} 2.02 mg/l CC LOE_bC 0.5 mg/l NC LC_{100} 10.0 mg/l NC	NC = nominal concentrations, CC = calculated concentration, test performed using Cremophor emulsifier; Noack, 1989
Acute toxicity to bluegreen algae, *Synechococcus* strain 6911, 24 h, nonstandard test	LOEC 3.0 mg/l NC	LOEC for growth rate, NC = nominal respectively loading concentration, saltwater; Juettner/BASF, 1982
Acute toxicity to bluegreen algae, Synechococcus strain 6911, 24 h, nonstandard test	LOErC 3.0 mg/l NC NOErC 2.0 mg/l NC	LOErC for growth and carotenogenesis, NC = nominal respectively loading concentration, saltwater; Jüttner & Bogenschütz, 1983
QSAR baseline algal EC50, TGD	EC50 1.13 mg/l CC	non-polar narcosis model, based on molecular weight and logPOW, CC = calculated concentration; following TGD, Commission of the European Communities, 1996
Inhibition of larval attachment to substrate, nonstandard	pseudoionone inhibits the attachment of marine larvae to substrate	at unspecified low concentrations; Japanese patent short text, no further information available, saltwater; Kuraray, 1982a
Toxicity to Artemia salina brine shrimp, nonstandard	possibly pseudoionone is toxic to brine shrimp	Japanese patent short text, no further information available, saltwater; Kuraray, 1982b

Fish toxicity

Kirsch and Munk [1989] reported an acute, static test over 96 hours with golden orfe *(Leuciscus idus)* according to DIN 38412, part 15. Even though this was not a GLP test, full details as to supplier, acclimatisation, animal husbandry, test procedures and statistics are given. Briefly, based on range-finding pretests with an LC_{50} of approximately 10 mg/l nominal concentration (NC), loadings of 100, 46.4, 21.5, 10.0 and 4.64 mg/l (all NC) or 0 mg/l (controls) were prepared by adding the corresponding amount of pseudoionone to the experimental tanks containing 10 l of equilibrated reconstituted freshwater according to DIN 38412, part 11, without any further pretreatment or emulsifier. Then, 10 fish were added per tank. They were observed at 1, 24, 48, 72 and 96

hours after introduction, any dead fish were removed and all signs, symptoms or other remarks recorded. At 24 hours, 7 fish were found dead at 10 mg/l NC and all 10 fish were dead at all higher concentrations; at 48 hours, all 10 fish were dead at 10 mg/l NC; at 96 hours, all fish at 4.64 mg/l NC and in the controls were still alive. Survivors in the 10 mg/l NC group at 24 hours showed signs of narcosis. The LC_0 in this test was 4.64 mg/l NC, the LC_{100} was 10 mg/l NC and the LC_{50} as determined by geometric mean was 6.8 mg/l NC; however, this derivation is not in the original report but was calculated from the data for the present SIDS and SIAR.

In support of this result, Gröner [1989] reported an acute, static fish test over 48 hours with rainbow trout *(Oncorhynchus mykiss)* for the chemically closely related test substance beta-ionone (CAS 14901–07–6). The author had routinely performed non-GLP biodegradation and fish tests as part of the internal substance documentation for the reporting company, F. Hoffmann-La Roche, for many years. While all reports are very short, listing only the results, test procedures basically followed the OECD 203 guideline, albeit fewer (3 or 4) concentrations were normally used based on range-finding pretests and test duration was only 48 hours. All fish at 5 mg/l NC survived but "showed effects", all fish were dead at 10 mg/l NC and the geometric-mean LC_{50} was 7.1 mg/l NC. This result is highly comparable to the pseudoionone data above.

For comparison and information on possible mechanisms of toxic action, the baseline, minimum or narcosis toxicity of pseudoionone to fish was calculated using a validated quantitative structure-activity relationship (QSAR) algorithm published in the EU Technical Guidance Document [TGD; Commission of the European Communities, 1996]. For fish toxicity and non-polar substances, the relationship is given as $\log LC_{50}$ (mol/l) = –0.85 log P_{OW} – 1.39, which results in a QSAR LC_{50} of 3.12 mg/l.

In conclusion, pseudoionone is acutely toxic to fish in the range of 1–10 mg/l, with an interpolated LC_{50} of 6.8 mg/l NC, an LC_0 of 4.64 mg/l NC and an LC_{100} of 10 mg/l NC. The closely related test substance beta-ionone showed highly comparable toxicity with 7.1, 5 and 10 mg/l NC, respectively. In both cases, all fish were dead at 10 mg/l NC and higher within 48 hours while all fish survived 4.64 or 5 mg/l NC, respectively. A comparison with a validated fish non-polar toxicity QSAR LC_{50} of 3.12 mg/l, which is in the same range as the experimental data, suggests narcosis or baseline/ minimum toxicity as the mechanism of action for pseudoionone in fish, which in turn makes a receptor-mediated mechanism of action unlikely.

Invertebrate toxicity

Noack [1990] reported an acute test with daphnids according to DIN 38412, part 11. While this was not a GLP test, full details as to supplier, acclimatisation, animal husbandry, test procedures and statistics are given. Briefly, *Daphnia magna* were exposed in daphnid medium according to DIN 38412, part 11, to pseudoionone. A 100-mg/l stock solution was prepared with water and Cremophor emulsifier at 10% of the pseudionone concentration, however, the reasons for the use of an emulsifier are not stated in the report. Based on a range-finding pretest showing toxicity below 10 mg/l NC, definitive test concentrations in quadruplicate were prepared with the stock solution diluted with daphnid medium at 0.58, 1.0, 1.8, 3.2, 5.8 and 10.0 mg/l NC as well as twice 0 mg/l (medium-blank and maximum-emulsifier control). Five young daphnids each were added to 50 ml of the respective solutions and inspected at 3, 6 24 and 48 hours after the start of the test. Immobilised animals were checked according to the DIN guideline and recorded. At 10 mg/l NC, 5% of daphnids were immobilised at 3 h, 20% at 6 h, 75% at 24 h and 100% at 48 h; at 5.8 mg/l NC, 45% at 24 h and 70% at 48 h; at 3.2 mg/l NC, 5% at 24 h and 50% at 48 h; at 1.8 mg/l NC, at 1.0 mg/l NC, 5% at 6 h without any more later; in the medium control without emulsifier, none of the daphnia became immobilised; while in the emulsifier control, 5% were immobilised at 24 and 48 h. The EC_{50} calculated by Spearman-Karber correlation was 3.7 (3.1–4.4, 95% confidence interval) mg/l NC while the calculated EC_0 was 1.0 mg/l NC. The 5% immobilised daphnids in the

1.0 mg/l NC concentration and in the emulsifier control both correspond to just one daphnia out of 20; as no daphnia became immobilised at the next higher concentration of 1.8 mg/l NC, this may be seen as either a chance event or possibly caused by the emulsifier. However, this is not discussed in the report.

For comparison and information on possible mechanisms of toxic action, the baseline, minimum or narcosis toxicity of pseudoionone to daphnia was calculated using a validated quantitative structure-activity relationship (QSAR) algorithm published in the EU TGD [Commission of the European Communities, 1996]. For daphnid toxicity and non-polar substances, the relationship is given as $\log LC_{50}$ (mol/l) = –0.95 log P_{OW} – 1.32, which results in a QSAR LC_{50} of 1.46 mg/l.

In conclusion, pseudoionone is acutely toxic to daphnids in the range of 1–10 mg/l, with an interpolated EC_{50} of 3.7 mg/l NC, a calculated EC_0 of 1.0 mg/l NC, a NOEC of 0.58 mg/l NC and an EC_{100} of 10 mg/l NC in a test using emulsifier; the NOEC may be misleadingly low due to the influence of the emulsifier or to a chance event. A comparison with a validated daphnid non-polar toxicity QSAR EC_{50} of 1.46 mg/l, which is in the same range as the experimental data, suggests narcosis or baseline/minimum toxicity as the mechanism of action for pseudoionone in daphnids, which in turn makes a receptor-mediated mechanism of action unlikely.

Two Japanese patent applications for an anti-fouling substance by Kuraray [1982a, 1982b] referring to invertebrate toxicity were located by searching for the CAS number of pseudoionone. Both are available only as the English abstracts, moreover, no further information was forthcoming on request. In one [1982a], probably pseudoionone (not specified in the text) at "low" concentrations inhibits the attachment of planktonic larvae of marine invertebrates to substrate. In the second [1982b], possibly pseudoionone (not specified in the text) is toxic to the marine brine shrimp, *Artemia salina*. Both sources cannot be critically judged and must therefore be seen at best as anecdotal evidence for toxicity of pseudoionone to marine invertebrates.

Algal toxicity

Noack [1989] reported a growth inhibition test with algae according to DIN 38412, part 9. This was not a GLP test, but full details as to supplier, algal culture, test procedures and statistics are given. Briefly, *Scenedesmus subspicatus* were exposed in algal medium according to DIN 38412 part 9, to pseudoionone in medium with Cremophor emulsifier at 10% of the pseudionone concentration. Again, the reasons for the use of an emulsifier are not stated in the report. Based on a range-finding pretest showing toxicity below 10 mg/l NC, definitive test concentrations in quadruplicate were prepared at 0.5, 1.0, 2.5, 5.0 and 10.0 mg/l NC as well as twice 0 mg/l (medium-blank and maximum-emulsifier control). The test was run over a total of 96 hours under illumination, the *p*H was determined in every single vessel at the start and at the end of the test, temperature was kept in the range of 21–25 °C. Density of algae was determined using fluorimetry at the start and then after every period of 24 hours. Additionally, fluorimetry showed that the emulsifier in the highest concentration had some autofluorescence, which was deducted from results, but that pseudoionone itself had no autofluorescence. There was no effect on *p*H that might have biased the results nor was there any influence on photosynthetic capacity of the algae. Detailed cell density results, averaged over the four replicates per concentration, are listed in the SIDS. The statistical evaluation showed the follwing results, all values in mg/l NC with 95% confidence interval in brackets: E_bC_{50}, 72 h 1.107 (0.37–3.29), 96 h 1.261 (0.48–3.32); E_bC_{10}, 72 h 0.525 (0.11–2.43), 96 h 0.625 (0.17–2.36); E_rC_{50}, 72 h 2.018 (0.71–5.76), 96 h 2.623 (1.21–5.67); E_rC_{10}, 72 h 1.085 (0.31–3.85), 96 h 1.655 (0.75–3.68). The measured LOEC was 0.5 mg/l NC and the measured EC_{100} was 10.0 mg/l NC. In summing up, pseudoionone with Cremophor as an emulsifier had a 72-hour E_bC_{50} of 1.1 mg/l NC and a 72-hour E_rC_{50} of 2.0 mg/l NC. Biomass was slightly but significantly inhibited at the lowest concentration tested (0.5 mg/l NC) while growth rate was not; 100% inhibition was seen at 10 mg/l NC. Over 96 hours, both E_bC_{50} and E_rC_{50} were slightly higher.

Additional inhibition data for bluegreen algae (Cyanobacteria) from nonstandard tests were briefly described by Juettner/BASF [1982] and Jüttner and Bogenschütz [1983]. The first author is the same person in both cases, the difference resulting from the German "ü" umlaut, which was not used in the first source; both publications describe the same experimental work. The first publication is very short, being a patent abstract. The second publication is listed in the SIDS under chapter 4.4, Toxicity to Micro-organisms, *e.g.,* Bacteria, due to bacterial toxicological endpoints being described. Briefly, cyanobacteria of the marine strain *Synechococcus* 6911 were incubated in a synthetic nutrient with test substance including pseudoionone at various final concentrations. After 30 minutes, 8, 16 and 24 hours of incubation the cell density per concentration respectively control was measured to determine the minimal inhibitory concentration or LOEC. Chlorophyll A and various carotenoids were determined after extraction and gel separation (full details in SIDS) to follow chlorophyll A biosynthesis, while growth was investigated for up to 180 hours. For pseudoionone, the NOEC for growth rate was 2 mg/l NC, while the LOEC was 3 mg/l NC. At 3 mg/l NC, growth as determined by optical density was indistinguishable from controls up to 24 hours, but then started to decline. Similarly, exposure to 3 mg/l did not have any influence on chlorophyll A biosynthesis up to 24 hours, the limit of this part of the test. However, the formation of carotenoid precursors was slightly reduced compared to controls already at 8 hours, with reduction becoming stronger over time. Specifically, at 3 mg pseudoionone/l NC, the biosynthesis of two intermediates, phytofluene and the subsequent zeta-carotene, reached a plateau at 16 respectively 24 hours; both substances were undetectable in exponentially growing control cultures due to immediate consumption in further biosynthesis. The reversibility of the inhibition of further carotenoid synthesis by pseudoionone was demonstrated when pseudoionone was washed out with new medium after a 30-hour incubation; growth rate and carotenoid synthesis re-approached that of control cultures and accumulated phytofluene and zeta-carotene were for the biggest part re-metabolised within 5–10 hours. In this test, 3 mg pseudoionone/l NC was the LOEC for both growth and biochemical parameters, the NOEC was 2 mg/l NC; the biochemical effects seen at 3 mg/l NC were reversible.

For comparison and information on possible mechanisms of toxic action, the baseline, minimum or narcosis toxicity of pseudoionone to algae was calculated using a validated quantitative structure-activity relationship (QSAR) algorithm published in the EU TGD [Commission of the European Communities, 1996]. For algal toxicity and non-polar substances, the relationship is given as $\log EC_{50}$ (mol/l) = –1.00 log P_{OW} – 1.23, which results in a QSAR EC_{50} of 1.13 mg/l.

In conclusion, pseudoionone is acutely toxic to algae in the range of 1–10 mg/l, with an interpolated 72-hour E_bC_{50} of 1.1 mg/l NC and a 72-hour E_rC_{50} of 2.0 mg/l NC, a biomass LOEC and growth-rate NOEC of 0.5 mg/l NC and an EC_{100} of 10 mg/l NC in a standard test using emulsifier. In a nonstandard test with marine cyanobacteria, the LOEC was 3 mg/l both for growth and biochemical endpoints, while the NOEC was 2 mg/l. This locates algal toxicity in the same range for both photosynthetic green algae and cyanobacteria. A comparison with a validated algal non-polar toxicity QSAR EC_{50} of 1.13 mg/l, which is in the same range as the experimental data, suggests narcosis or baseline/minimum toxicity as the mechanism of action for pseudoionone in algae, which in turn makes a receptor-mediated mechanism of action unlikely.

Chronic Toxicity Test Results

No proper chronic ecotoxicity data have been located. However, the biomass LOEC and growth rate NOEC in the DIN 38412 algal growth test was 0.5 mg/l NC while the NOEC in the marine cyanobacterial test was 2 mg/l.

Toxicity to Micro-organisms

Several results from tests with micro-organisms have been located for pseudoionone. A bacterial respiration inhibition test was performed according to OECD 209 and ISO 8192 with pseudoionone

[Pagga, 2002b; original test conducted in 1988], comparing the baseline oxygen consumption of activated sludge from a municipal wastewater treatment plant to that of the same sludge with added pseudoionone over 30 minutes. The blank respiration rate after 30 minutes was 26 mg/(l×h) while for pseudoionone the extrapolated 30-minute values were: EC_{20} ~ 300 mg/l NC, EC_{50} and EC_{80} > 1000 mg/l. In conclusion, pseudoionone has a high toxic threshold concentration, defined as the EC_{20}, of approximately 300 mg/l NC.

In support of this finding, the closely related substance beta-ionone (CAS 14901–07–6) at 30 mg/l did not cause toxicity to activated sludge nor inhibition of co-metabolic substrate degradation in an respirometric inherent biodegradation test [Gröner, 1989].

The growth inhibition test with marine cyanobacteria with pseudoionone described above [Jüttner and Bogenschütz, 1983] resulted in a LOEC for both growth and biochemical parameters of 3 mg pseudoionone/l while the NOEC was 2 mg/l; the biochemical effects seen at 3 mg/l were reversible.

In an ultimate anaerobic biodegradation test according to ISO 11734 [Häner, 2002], the calculated net inorganic carbon (IC) production in the test flasks was consistently negative after substraction of the blank control IC for the same sampling points. Hence, pseudoionone was inhibitory respectively toxic to anaerobic bacteria at the test concentration of 122 mg/l.

4.2 Terrestrial Effects

Scattered information of variable reliability is available for pseudoionone. No data for birds, reptiles or amphibians have been located.

Chronic Toxicity Test Results

A growth and fertility test with pseudoionone with the common soil and sediment nematode *Caenorhabditis elegans* was performed by Höss [2002]. *Caenorhabditis* are mostly self-fertilising hermaphrodites that pass through four juvenile stages with moults to reach adult stage, self-fertilise and develop eggs in their body; a full reproductive cycle takes about 72 hours at room temperature. The test performed corresponds to the recent DIN draft, with the exception of a shorter overall duration (72 instead of 96 hours). However, in view of the short reproduction time of *Caenorhabditis,* this test qualifies as a chronic study. Briefly, synchronised juvenile *Caenorhabditis* of the first stage (J1) were used for the test in artificial sediment containing M9-medium (full details in SIDS). Pseudoionone was dissolved in an ethanol concentration series and 0.01 ml of the respective stock solution was thoroughly mixed with 0.75 g wet artificial sediment in polystyrene multiwell test vessels; then spiked sediments were left for 24 hours to allow equilibration of test substance between aqueous and solid phases. Before the start of the assay, 0.25 ml of *Escherichia coli* bacterial suspension in double-concentrated M9-medium was added to each test well as food. Then, 10 stage J1 worms were added to each well. Every test concentration including a vehicle control was run in triplicate for the range-finding test and in quintuplicate for the main test. The multiwell plates were incubated for 72 hours on a shaker at ±20 °C. To stop the test, nematodes were heat-killed by warming the plates to approximately 55 °C, which makes them stretch, and stained with Rose Bengal dye. Nematodes were extracted from the sediment by centrifugation in a density gradient and parameters for the endpoints were determined under a microscope. The parameters for the endpoints were as follows. Growth: length in µm; egg production: number of eggs in body; fertility: percentage of gravid worms (worms with ≥ 1 egg). For statistical evaluation, one-way ANOVAs, Dunnett *post-hoc* tests and sigmoidal dose-response curves for the determination of EC_x values were used.

The range-finding pretest had shown no effect up to 100 mg/kg sediment (dry weight). In the main test, there was a significant reduction in growth (–15.3%) and egg production (–38.8%) at 200 mg/kg sediment, while fertility as measured by number of gravid worms was only significantly

reduced (–55.9%) at 800 mg/kg sediment. Observed and interpolated effect concentrations in mg/kg sediment (dry weight) are as follows. Growth: NOEC = 100, LOEC = 200, EC_{50} = 2490 and EC_{90} = 5183. Egg production: NOEC = 100, LOEC = 200, EC_{50} = 821 and EC_{90} = 2893. Fertility: NOEC = 400, LOEC = 800, EC_{50} = 1537 and EC_{90} = 3193. The NOEC for pseudoionone was 100 mg/kg sediment (dry weight) for growth and egg production and 400 mg/kg for fertility. While effects on growth, egg production or fertility were observed at higher concentrations, the concentration-effect curves for all three parameters show a relatively flat slope. Hence, pseudoionone is of low toxicity to soil- and sediment-dwelling nematodes, with a chronic NOEC of 100 mg/kg (dry weight) and the lowest EC_{50} on egg production of 821 mg/kg (dry weight).

Toxicity to Insects

Insects grow through one or more developmental stages; their moulting system is under control of two hormones, ecdysone (which initiates moulting) and juvenile hormone (which inhibits moulting). As the latter has a terpenoid skeleton, many natural and synthetic terpenoid compounds including pseudoionone have been investigated for insect-toxic properties and potential use as pesticides.

In a British patent specification by Pfizer Ltd [1972], pseudoionone was applied topically to the venter of 20 yellow meal worm (*Tenebrio molitor*) pupae of 48 hours age, as a single dose of up to 1000 µg per pupa. Two zero-dose controls were run, solvent-only and no treatment at all. After application the pupae were kept singly in glass beakers in a temperature-controlled chamber at high humidity for 7 days, when the number of normally metamorphosed mealworms or no or only partial metamorphosis was determined. Pseudoionone affected moulting dose-dependently between 7.81 µg/pupa (0% inhibition) and 250 µg/pupa (100% inhibition), full details are listed in the SIDS. The solvent control interfered with moulting in 10.5% of cases while the blank control had no effect at all. By crude visual interpolation on a log graph (not in the source, made during preparation of the SIDS), the EC_{50} corresponds to a dose of ~ 64 µg/pupa. Hence, pseudoionone has a certain juvenile-hormone-like effect on mealworm pupae.

Kuziak and colleagues [1978] tested 33 different terpenoid compounds including pseudoionone for juvenile hormone activity on larval or juvenile stages of four different insects, namely *Dysdercus cingulatus* (red cotton bug), *Tenebrio molitor* (yellow mealworm), *Musca domestica* (housefly) and *Aedes aegypti* (yellow fever mosquito). Briefly, solutions of the test substances in various concentrations were applied topically by a droplet of 1 µl on the cuticle of newly moulted larvae of *D. cingulatus,* pupae of *T. molitor* and both larvae and pupae of *M. domestica*. Controls were treated with 1 µl of solvent. For *A. aegypti,* test substances were added to food for third and fourth larval stages at a maximal concentration of 10 mg/l food, controls received food with added solvent. The biological activity was determined by estimating the dose needed for 50% inhibition (ID_{50}) of metamorphosis. Pseudoionone did not have any juvenile hormone activity at the highest concentrations on *D. cingulatus* (80 µg/specimen) or on *M. domestica* (10 mg/specimen both for larvae and pupae). In contrast, pseudoionone showed an ID_{50} for metamorphosis at the highest concentration in *T. molitor* (80 µg/specimen). Moreover, pseudoionone was toxic to *A. aegypti* with an LC_{50} of 10.15 µg/l diet. Within a group of six pseudoionone analogues, pseudoionone itself showed the lowest relative juvenile hormone activity regarding *T. molitor, D. cingulatus* and *M. domestica;* it also had the lowest toxicity for *A. aegypti.* In comparison with other, non-pseudoionone-analogue compounds, pseudoionone showed both low relative inhibition of metamorphosis and low toxicity against *A. aegypti.* Hence, in a comparative study on four insect species, pseudoionone showed limited juvenile hormone activity and low toxicity.

Slama [1978] reported the effects of various test substances including pseudoionone on larval development in *Pyrrhocoris apterus* (fire bug). Solutions of test substances were either applied topically to the cuticle of the larvae or indirectly on the filter paper substrate of the larvae. The following parameters were recorded: 1) the length of the intercdysial period, indicating disturbances

in the moulting cycles; 2) qualitative changes in the succession of the larval instars, *i.e.,* prothetely and metathely in technical terms; and 3) local prothetelies or metathelies, indicating developmental disproportions between the different tissues of the larvae. Anti-ecdysone-like activity was noted for pure pseudoionone as an "antifeeding effect associated with reversible inhibition of larval development. The effects were characterised by suppressed or arrested feeding, though the food [itself] was not directly contaminated, decreased water uptake, prolonged interecdysial periods if ecdysis was at all evident, incomplete coordination of the locomotion and decreased survival. Specimens which overcame the ecdysial failures gave rise to extremely small adults with rudimentary wings." Hence, at a dose of probably 10 µg/larva (not explicitly stated, highest dose) and possibly both by direct and indirect application, pseudoionone had an anti-ecdysone-like activity on larvae of *P. apterus*.

Mehta [1979] tested 16 terpenoids for their effect on embryonic development in the moth, *Earias vittellata.* Test compounds dissolved in isopropyl alcohol were spread in different concentrations on the bottom of glass tubes. Eggs of *E. vittellata* in groups of 20 were placed in contact with the respective test compound in the glass tubes and examined daily for the number of eggs hatched. Appropriate controls were run and each experiment was replicated five times. The effect of terpenoids was expressed in term of per cent inhibition of embryonic development, normalised to hatching success in the controls. In contrast to other terpenoids, pseudoionone did not inhibit the development of *E. vittellata* eggs even at the highest exposure concentration of 133.2 µg/cm^2 tube surface.

Atkins and co-workers [1975] tested various substances in the laboratory as potential honeybee-repellent additives to pesticides in order to reduce pesticide hazards to honeybees. In the gustatory test, where gustatory repellence and oral toxicity were assayed, the test substances were prepared as 1% stock solutions. Serial dilutions were incorporated to 1:1 honey-water feeding mixtures, filled in vials that allowed the bees to feed. A similar vial, but without test substance in the feeding solution, was offered as an alternative, control feeding station. In the gustatory test table, (E)-pseudoionone is listed as nontoxic, which is interpreted that no bees died during the 24 hours of exposure. Hence, at unspecified concentrations <1% pseudoionone was not orally toxic to honeybees over 24 hours.

In conclusion, pseudoionone showed juvenile-hormone-like activity in a number of insect species at a topically applied dose of 10–80 µg per larva or pupa or through cuticular uptake from substrate. In comparison with other terpenoids, however, this effect was relatively weak. Subsequent to dietary uptake, pseudoionone was toxic to mosquito larvae with an LC_{50} of 10.15 µg/l diet; in contrast, it was not toxic to honeybees at at unspecified concentrations <1% in food.

Toxicity to Terrestrial Plants

Pseudoionone has been detected in a number of flowering plants (see chapter 2.1.3). It was made likely that pseudoionone is both a precursor in the biosynthesis and a metabolite in the degradation of lycopene, a common carotenoid, which in turn would make pseudoionone a very common but probably short-lived intermediate in plant metabolism.

By searching for the CAS number 141–10–6, a Japanese patent abstract [Kuraray, 1981] for an agricultural fungicide was located. While the available English text does not explicitly list pseudoionone, it is said that the “agricultural fungicide contains terpene type carbonyl cpd. of formula (I), (II) or (III) [no further information available] … The active cpd. can be used for the protection of paddy rice, upland crops, fruit trees and wood from the attack of pathogenic fungi … The active cpd. shows excellent effect in the control of rice blast and rice helminthosporium leaf spot, and has no phytotoxicity to rice and other crops.” No further details were forthcoming on request. Hence, while probably pseudoionone in combination with other compounds may work as a fungicide at unstated concentrations, it may be nontoxic to rice, other crops and trees.

Toxicity to Micro-organisms

Mickinney and co-workers [1952] investigated the effect of test substances including pseudoionone on growth and carotenoid biosynthesis of the mould *Phycomyces blakesleeanus.* Briefly, aliquots of test substance were added to standardised pre-grown cultures, with thick but short aerial mycelium, negligible pigmentation and no fruiting bodies, which were subsequently kept in the dark for 24 hours. The end points were further growth, development of fruiting bodies and of pigmentation as determined by colour. At ~ 220 mg/l, pseudoionone clearly inhibited the growth of *P. blakesleeanus* cultures; at ~ 22 mg/l, growth was nearly normal and overall pigment production was nearly as high as in controls.

Maruzzella and colleagues [1961] tested the vapours of 196 chemicals including pseudoionone *in vitro* against growing fungal cultures of *Candida albicans*, *Phoma betae*, *Geotrichum candidum* and *Oospora lactis*. Briefly, Sabouraud maltose agar was poured into Petri dishes, allowed to harden and seeded with the respective test organism from a mature broth culture. Small aluminium cups containing 0.5 ml of test substance were placed in the centre of the Petri dish top, then the seeded agar-plated bases were inverted on the tops, so that the agar surface was above the sample. Vapours of the chemicals were then allowed to emanate for a 5-day incubation period at 22 °C. Chemicals were tested in triplicate. A clear growth inhibition zone on the agar surface indicated antifungal activity of the vapour, the larger the zone the greater the activity. With pseudoionone, there was no inhibition of *C. albicans, G. candidum* and *O. lactis* at all, while only *P. betae* showed an inhibition zone diameter of 20 mm. Comparing the fungi for sensitivity towards all test substances, *P. betae* was the most sensitive overall while all others showed lower but comparable sensitivity.

A Canadian patent application for a “veterinary disinfectant containing ionone and terpene” [Franklin *et al.,* 1996] describes a mixture that “comprises about 45% ionone, about 40% another terpene, about 20% surfactant, and about 5% iso-Pr alc”. This preparation is “effective against several types of bacteria and a broad range of fungi, and is esp. useful in veterinary medicine for control of foot diseases. […] As a foot bath, the compn. is dild. with water about 1 to 1,000, and as a spray, it is dild. with water or org. solvent about 1:1 to 1:100. Preferred ionones are beta-ionone and pseudoionone.” In this publication that was only seen as the abstract, pseudoionone (in a mixture with other terpene, surfactant and isopropanol) is described as an antibacterial and antifungal compound, with active concentrations between 45% and 0.045%, discounting the effect of the other ingredients.

In the above Japanese patent abstract [Kuraray, 1981] for an agricultural fungicide, the “active cpd. [which probably includes pseudoionone, as the source was found by searching for the CAS number] shows excellent effect in the control of rice blast and rice helminthosporium leaf spot …” No further details were forthcoming on request. Hence, possibly pseudoionone in combination with other terpene compounds at unstated concentrations may work as a fungicide for crops and trees.

In conclusion, in the detailed sources pseudoionone did show some, however limited, toxicity to fungi. Not all species were susceptible and in one that was, the LOEC is 22 mg/l medium, which is not considered highly toxic. Two other sources describe mixtures of pseudoionone with other substances that have antifungal properties; in one of those source, discounting the effect of the other ingredients, the active pseudoionone concentrations are given as > 450 mg/l. Based on these data, pseudoionone is judged to have a limited toxic potential against fungi.

4.3 Other Environmental Effects

No data located.

4.4 Initial Assessment for the Environment

In the aquatic compartment, pseudoionone has a moderate potential for toxicity with all EC_{50} or LC_{50} values for fish, daphnia, green algae and cyanobacteria consistently in the range of 1–10 mg/l. QSAR modelling suggests baseline toxicity ("narcosis") as the mechanism of action, there is no reason to assume that pseudoionone acts on specific receptors in the above groups. According to two vague sources, pseudoionone at unspecified "low" concentrations may inhibit the attachment of larvae of various marine invertebrate groups to substrate and may show toxicity against brine shrimps.

In a standard activated sludge respiration inhibition test, pseudoionone had a high toxic threshold concentration, defined as the EC_{20}, of approximately 300 mg/l while the EC_{50} was > 1000 mg/l. Tests with cyanobacteria resulted in a NOEC of 2 mg/l. In contrast, at 122 mg/l, pseudoionone was toxic to anaerobic bacteria from a sludge digester. Also, pseudoionone was described as an ingredient in a veterinary disinfectant formulation with antifungal and antibacterial properties at higher concentrations.

Regarding insect toxicity, pseudoionone showed limited juvenile-hormone-like activity in a number of species by cuticular uptake. However, in comparison with other terpenoids this effect was relatively weak. Pseudoionone was toxic to mosquito larvae by oral uptake with an LC_{50} of 10.15 µg/l diet but it was not toxic to honeybees at concentrations < 1% in food.

In a chronic toxicity test with the common nematode *Caenorhabditis elegans* in artificial sediment, pseudoionone showed low toxicity to a sediment- and soil-dwelling organism, with a consistent NOEC of 100 mg/kg (dry weight) for three different chronic endpoints. Based on few available data, pseudoionone is judged to have at worst a limited toxic potential against fungi. Last, the reported occurrence in various plants is interpreted as evidence for pseudoionone being a common but probably short-lived intermediate in plant metabolism; in possible confirmation, a single vague source suggests that probably pseudoionone is nontoxic to various flowering plants.

In conclusion, the reported toxicity of pseudoionone to environmental species is mostly moderate or low, with effective concentrations > 1 mg/l in the aquatic compartment. There is some evidence for toxicity at higher concentrations to anaerobic bacteria, fungi, possibly insects and possibly marine invertebrates or their larvae. However, no single result points at an elevated ecotoxicological potential.

5 RECOMMENDATIONS

The chemical is currently of low priority for further work.

5.1 Rationale for the recommendations

Human health: The only hazards identified is irritation to skin and slight irritation to eyes as well as sensitisation. 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.

Environment: The chemical possesses properties indicating a hazard for the environment. Based on data presented by the Sponsor country, exposure to the environment 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.

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I U C L I D

D a t a S e t

Existing Chemical	ID: 141-10-6
CAS No.	141-10-6
EINECS Name	6,10-dimethylundeca-3,5,9-trien-2-one
EC No.	205-457-1
Generic name	Pseudoionone
Molecular Formula	C13H20O

Producer Related Part	
Company:	Hoffmann-La-Roche AG
Creation date:	01-OCT-2002

Substance Related Part	
Company:	Hoffmann-La-Roche AG
Creation date:	01-OCT-2002

Memo:	ICCA HPVC Initiative/OECD SIDS; correct company name is F. Hoffmann-La Roche AG, Basel

Printing date:	10-JAN-2006
Revision date:	
Date of last Update:	**10-JAN-2006**

Number of Pages:	**139**

Chapter (profile):	Chapter: 1, 2, 3, 4, 5, 6, 7, 8, 10
Reliability (profile):	Reliability: without reliability, 1, 2, 3, 4
Flags (profile):	Flags: without flag, confidential, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

1.0.1 Applicant and Company Information

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30-DEC-2002

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21-JAN-2003

Type: cooperating company
Name: BASF AG
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30-DEC-2002

1.0.2 Location of Production Site, Importer or Formulator

Type: manufacturer
Name of Plant: Teranol AG, Lalden
Street: PO Box 310
Town: CH-3930 Visp
Country: Switzerland
Phone: +41 279 485 733
Telefax: +41 279 486 184

30-DEC-2002

1.0.3 Identity of Recipients

1.0.4 Details on Category/Template

1.1.0 Substance Identification

IUPAC Name: 3,5,9-undecatrien-2-one, 6,10-dimethyl-
Smiles Code: CC(=O)C=CC=C(C)CCC=C(C)C
Mol. Formula: C13-H20-O
Mol. Weight: 192.30

Remark: Pseudoionone (CAS 141-10-6) is an acyclic C13 ketone with a terpenoid skeleton. It is a mixture of cis-2-pseudoionone (CAS 33073-35-7) and trans-2-pseudoionone (CAS 3796-54-1) with a slight preponderance of the cis isomer, due to the method of manufacturing.
Reliability: (1) valid without restriction
Peer-reviewed database published by the American Chemical Society that is responsible for the CAS numbering system and database.
06-JUN-2003 (8) (27) (87) (101)

1.1.1 General Substance Information

Purity type: other: Specifications
Substance type: organic
Physical status: liquid
Purity: >= 90 - % w/w
Colour: yellow, clear
Odour: "characteristic"

Remark: Pseudoionone is an intermediate in the synthesis of certain carotenoids, vitamins A, E and K1 as well as certain terpenoids. It is not used a such in final products.
The specification of minimum 90% is for the sum of cis- and trans-pseudoionone isomers.
08-JAN-2003 (27) (97)

Purity type: measured for specific batch
Substance type: organic
Physical status: liquid
Purity: = 96.1 - % w/w
Colour: yellow, clear
Odour: not stated

Result:

Composition	Result	Specification	Method
Pseudoionone	96.1%	>= 90.0 % w/w	GC
Pseudoionone isomers 1+2	2.2%	<= 2.5 % w/w	GC
6-Methylhept-5-en-2-one	< 0.03%	<= 1.0 % w/w	GC
3,7-Dimethyloct-6-en-1-yn-3-ol	< 0.03%	<= 1.5 % w/w	GC
C16-components	0.83%	<= 3.5 % w/w	GC
Sum, other impurities	0.89%	<= 3.5 % w/w	GC
Aspect	liquid	liquid	visual
	clear	clear	visual
Colour	yellow	yellow	visual

Passed 28-Mar-2002
Reliability: (2) valid with restrictions
30-DEC-2002 (96)

1.1.2 Spectra

1.2 Synonyms and Tradenames

Citrylideneacetone

30-DEC-2002 (87)

Pseudo-ionone

06-JUN-2003 (87)

2,6-Dimethyl-2,6,8-undecatrien-10-one

30-DEC-2002 (87)

2,6-Dimethylendeca-2,6,8-trien-10-one

30-DEC-2002 (87)

2-Pseudoionone

07-JAN-2003 (7)

3,4-Dehydrogeranylacetone

15-JAN-2003 (2)

6,10-Dimethyl-3,5,9-undecatrien-2-one

30-DEC-2002 (87)

9-Apo-psi-caroten-9-one

14-JAN-2003 (50)

psi-Ionone

06-JUN-2003 (87)

1.3 Impurities

Purity type: other: Specifications
CAS-No: 110-93-0
EC-No: 203-816-7
EINECS-Name: 6-methylhept-5-en-2-one
Mol. Formula: C8-H14-O
Contents: <= 1 - % w/w

08-JAN-2003 (27)

Purity type: other: Specifications
CAS-No: 29171-20-8
EC-No: 249-482-6
EINECS-Name: 3,7-dimethyloct-6-en-1-yn-3-ol
Mol. Formula: C10-H16-O
Contents: <= 1.5 - % w/w

08-JAN-2003 (27)

Purity type: other: Specifications

EINECS-Name: pseudoionone, other isomers
Mol. Formula: C13-H20-O
Contents: <= 2.5 - % w/w

08-JAN-2003 (27)

Purity type: other: Specifications
EINECS-Name: "C16-components", different isopropylidene-substituted pseudoionone compounds
Mol. Formula: C16-H24-O
Contents: <= 3.5 - % w/w

08-JAN-2003 (27)

Purity type: other: Specifications
EINECS-Name: sum, other (undefined) impurities
Contents: <= 3.5 - % w/w

08-JAN-2003 (27)

1.4 Additives

EINECS-Name: none

08-JAN-2003 (27)

1.5 Total Quantity

Quantity: = 10000 - 50000 tonnes produced in 2002

08-JAN-2003 (95)

1.6.1 Labelling

1.6.2 Classification

Classified: provisionally by manufacturer/importer
Class of danger: other: irritating, dangerous for the environment
R-Phrases: (38) Irritating to skin
(43) May cause sensitization by skin contact
(51/53) Toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment

05-JUN-2003 (28) (49)

1.6.3 Packaging

1.7 Use Pattern

Type: industrial
Category: Chemical industry: used in synthesis
08-JAN-2003 (95)

1.7.1 Detailed Use Pattern

Industry category: 3 Chemical industry: chemicals used in synthesis
Use category: 41 Pharmaceuticals
Extra details on use category: No extra details necessary
No extra details necessary
Emission scenario document: not available
Fract. of tonnage for application: .7
Fract. of chemical in formulation: 1
Production: yes
Processing: yes

Remark: Approximately 70% of pseudoionone produced is used in the synthesis of mainly vitamin E (dl-alpha-tocopherol and its esters) and to a smaller extent of vitamin A (retinoic acid and its esters), for food and feed fortification and, in the case of vitamin A products, as pharmaceutical specialities. Pseudoionone itself is not used as such in the formulation of food or feed fortification nor of pharmaceutical products.
15-JAN-2004 (95)

Industry category: 3 Chemical industry: chemicals used in synthesis
Use category: 26 Food/feedstuff additives
Extra details on use category: No extra details necessary
No extra details necessary
Emission scenario document: not available
Fract. of tonnage for application: .25
Fract. of chemical in formulation: 1
Production: yes
Processing: yes

Remark: Approximately 25% of pseudoionone produced is used in the synthesis of certain carotenoids, eg, apocarotene, apocarotenoic ester, beta-carotene, canthaxanthin or lycopene, which are formulated as food and feed additives. Pseudoionone is not used as such as a food or feed additive.
15-JAN-2004 (95)

Industry category: 3 Chemical industry: chemicals used in synthesis
Use category: 36 Odour agents
Extra details on use category: No extra details necessary
No extra details necessary
Emission scenario document: not available
Fract. of tonnage for application: .05
Fract. of chemical in formulation: 1
Production: yes

Remark: Approximately 5% of pseudoionone produced is used in the chemical synthesis of terpenoid substances, mainly in the fragrance area, a smaller part in the synthesis of flavour substances. Pseudoionone itself is not used as such as a fragrance or a flavour substance.
15-JAN-2004 (95)

Industry category: 5 Personal / domestic use
Use category: 26 Food/feedstuff additives
Extra details on use category: No extra details necessary

No extra details necessary
Emission scenario document: not available
Processing: yes
Private use: yes

Remark: Pseudoionone is a registered (and therefore accepted) food flavouring substance in the EU. However, actual use data for this application could not be retrieved but, based on Roche data, this use must be limited to much less than 0.1% of total production.
Reliability: (2) valid with restrictions
10-JAN-2006 (19)

1.7.2 Methods of Manufacture

Orig. of Subst.: Synthesis
Type: Production

Result: Total chemical synthesis of pseudoionone may start from the addition of acetylene (CAS 74-86-2) to acetone (67-64-1) resulting in 3-methyl-1-butyn-3-ol (115-19-5), which is hydrated in the presence of a palladium catalyst to 3-methyl-1-buten-3-ol (115-18-4), which is reacted with either diketene or acetic acid ester to the acetoacetate and the latter thermally reacted to 2-methyl-2-hepten-6-one (110-93-0). Alternatively, 3-methyl-1-buten-3-ol is reacted with isopropenyl methyl ether (116-11-0) to 2-methyl-2-hepten-6-one. In a third synthetic pathway, isoprene hydrochloride is reacted with acetone in the presence of an alkaline condensating agent or in the presence of organic bases as catalysts to 2-methyl-2-hepten-6-one. 2-Methyl-2-hepten-6-one is then reacted with acetylene to dehydrolinalool (29171-20-8), to which isopropenyl methyl ether is added to make pseudoionone (141-10-6). Alternatively, 3,7-dimethyl-2,6-octadienal (citral, 5392-40-5; two isomers, citral a = geranial, 141-27-5, and citral b = neral, 106-26-3) is condensed with acetone (67-64-1) to pseudoionone.
04-JUN-2003 (8) (29) (32) (66) (83) (101)

1.8 Regulatory Measures

Legal Basis: other: EU Directive 76/768/EEC (Cosmetics Directive)
Type of Meas.: Banned

Result: According to the International Fragrance Association [IFRA, 1979] and the EU Scientific Committe on Cosmetics Products and Non-Food Products [SCCNFP, 2000a, 2000b], pseudoionone (among other substances) should be banned from use as a fragrance in cosmetics products within the EU, based on sensitisation data published in the Monographs on Fragrance Raw Materials (Fd Chem Toxicol, 26(4): 311-312, 1988). However, pseudoionone and pseudomethylionone may be present as impurities in various ionones at an upper limit of 2%.
18-NOV-2003 (47) (85) (86)

1.8.1 Occupational Exposure Limit Values

Type of limit: other: none established.

08-JAN-2003

1.8.2 Acceptable Residues Levels

Proposed residues level: Residue in ionone fragrance compounds
Maximum residues level: 20 mg/kg

Result: According to the SCCNFP, pseudoionone (among other substances) should be banned from use as a fragrance in cosmetics products within the EU, based on sensitisation data published in the Monographs on Fragrance Raw Materials (Fd Chem Toxicol, 26(4): 311-312, 1988). Pseudoionone and pseudomethylionone may be present as impurities in various ionones at an upper limit of 2%.

20-JAN-2003 (85) (86)

1.8.3 Water Pollution

Classified by: other: German Verwaltungsvorschrift wassergefährdende Stoffe, VwVwS of 17-MAY-1999
Labelled by: other: own classification and labelling based on criteria in VwVwS, Annex 3
Class of danger: 2 (water polluting)

Result: Based on the current own (ie, non-Annex I) EU classification (Xi, N, R38-43-51/52, S24-37-61) for pseudoionone and the criteria and rules set out in Annex 3 to the German VwVwS of 1999, Pseudoionone is to be classified and labelled in Germany as water hazard class 2 ("dangerous to water").

15-JAN-2004 (28) (102)

1.8.4 Major Accident Hazards

1.8.5 Air Pollution

1.8.6 Listings e.g. Chemical Inventories

Type: AICS
Additional Info: Australian Inventory of Chemical Substances, June 1996 ed.

06-JUN-2003 (87)

Type: DSL
Additional Info: Canadian Domestic Substances List, Supplement to Canada Gazette, Part I, January 26, 1991

06-JUN-2003 (87)

Type: EINECS
Additional Info: European INventoy of Existing Chemical Substances, Annex to

Official Journal of the European Communities, 15 June 1990; EINECS no. 205-457-1

06-JUN-2003 (87)

Type: ENCS
Additional Info: Japanese Existing and New Chemical Substances List, Japanese Gazette; contained within class: low molecular chain-like organic compounds; ENCS no. 2-569.

06-JUN-2003 (87)

Type: ECL
Additional Info: Korean Existing Chemicals List, January 1997; ECL serial no. KE-11898.

06-JUN-2003 (87)

Type: PICCS
Additional Info: Philippine Inventory of Chemicals and Chemical Substances, 2000.

06-JUN-2003 (87)

Type: TSCA
Additional Info: US Toxic Substances Control Act; US HPVC94 Additions

06-JUN-2003 (87) (103)

Type: other: EU Register of flavouring substances used in or on foodstuffs
Additional Info: Listed name: Pseudo-ionone, CoE no. 11191.

Remark: Pseudoionone is a registered (and therefore accepted) flavour substance in the European Union.
06-JUN-2003 (19) (87)

1.9.1 Degradation/Transformation Products

Type: degradation product
CAS-No: 1604-28-0
EC-No: 216-507-7
EINECS-Name: 6-methylhepta-3,5-dien-2-one
IUCLID Chapter: 1.11

Result: Degradation product of pseudoionone in air-saturated water after 3 hours at 97 °C.
04-JUN-2003 (53) (54)

1.9.2 Components

1.10 Source of Exposure

Source of exposure: Human: exposure by production
Exposure to the: Substance

Result: The whole synthesis of pseudoionone, including the last reaction of dehydrolinalool (CAS 29171-20-8) with isopropenyl methyl ether (116-11-0) to pseudoionone, takes place in a closed, dedicated system. This system is only opened for the following activities: sampling for analyses (approximately 12 samples are taken per day through a small sampling port) and trouble-shooting respectively fault repairs involving opening of the closed system.
Exposure
For sampling, due to the size of the sampling port and the sample itself, exposure is minimal and it is further diminished through a high rate of air change in the production building. In case of repairs, parts of the system are isolated and flushed before repairs or exchange of parts or in-process control equipment, thanks to the flushing and air change rate exposure is again low. Filling of pseudoionone into barrels for transport takes place under a local exhaust, filling of road and rail transport containers takes place using a pivot-mounted filling installation. Gaseous emissions from the air changes in the building, from the local exhaust and from the pivot arm are bled into the atmosphere. Comparable technical installations apply both at the plant of the co-sponsor and co-producer BASF and at the recipient plants.
Production workers wear protective overall, safety work boots, nitrile-rubber gloves and saftey goggles. During more than 30 years of pseudoionone production at the Teranol Lalden plant, no effects of work-related exposure to pseudoionone have become registered.

Conclusion: Production worker exposure to pseudoionone is minimised through closed systems with limited (planned) breaching, air change rate in the production building, local exhausts during manned filling and open-air filling of large tranport containers. Workers wear standard chemical protection gear and are instructed, during more thatn 30 years of production at teranol Lalden, no effects related to pseudoionone exposure have become known.

Reliability: (1) valid without restriction
Overview by production site Safety and Environment Officer with many years of experience, based on his internal notes and reports. Reliability 1.

16-JUN-2003 (41)

Source of exposure: Environment: exposure from production
Exposure to the: other: Substance plus educts and impurities

Result: The whole synthesis of pseudoionone, including the last reaction of dehydrolinalool (CAS 29171-20-8) with isopropenyl methyl ether (116-11-0) to pseudoionone, takes place in a closed, dedicated system. After this last reaction step, both excess isopropenyl methyl ether and the reaction by-product, dimethoxypropane (CAS 77-76-9), are separated, the pseudoionone is transferred into another solvent, extracted and purified through distillation, still in a closed system.

Gaseous and liquid emissions
The gaseous emissions of the single production systems in the Teranol Lalden plant are collected and incinerated. Because of the collection, the single emissions from pseudoionone are not analysed, however, based on a quantitative estimation and a worst-caseoff-gas incineration failure rate of 5%, the resulting total volatile organic carbon (VOC) emissions from the last step of pseudoionone synthesis, including pseudoionone but also other educts, by-product, solvents and impurities, is estimated to be less than 0.05 kg VOC/t pseudoionone.
The total organic carbon (TOC) of the aqueous liquid emissions from the extraction and distillation was analytically determined as 5.8 kg TOC/t pseudoionone. This wastewater has been tested as well inherently biodegradable (>98% elimination) and is treated with other production wastewater streams in the regional mixed industrial/domestic sewage works.
Distillation residues correspond to approximately 100 kg of combustible organic waste per tonne of pseudoionone, which is incinerated in the in-house incineration plant, together with the gaseous waste streams.
Based on information from the co-sponsor BASF, the same synthesis with highly comparable sources and also treatments of emissions also holds for the German co-producer of pseudoionone.

Conclusion: Apart from limited emissions into the air during filling of containers and repair/maintenance work, all waste streams into the environment are captured and treated through incineration or wastewater treatment. Total emission of pseudoionone into the environment from the production site is very low.

Reliability: (1) valid without restriction
Overview by production site Safety and Environment Officer with many years of experience, based on his internal notes and reports. Reliability 1.

10-JAN-2006 (5) (41)

1.11 Additional Remarks

Memo: Natural occurrence

Result: Pseudoionone has been identified in several plants. The following list is illustrative but not exhaustive.

Species	Family	Common name
Aspalathus linearis	Fabaceae	rooibos/redbush
Campsis grandiflora	Bignoniaceae	-
Cassia acutifolia	Fabaceae	-
Cassia angustifolia	Fabaceae	-
Glycyrrhiza glabra	Fabaceae	licorice
Ilex paraguayensis	Aquifoliaceae	mate
Iochroma gesnerioides	Solanaceae	-
Leea guineensis	Leeaceae	-
Lycium halimifolium	Solanaceae	common matrimony vine
Lycopersicon esculentum	Solanaceae	tomato
Lysimachia capillipes	Primulaceae	-
Nicotiana tabacum	Solanaceae	(American) tobacco

Passiflora edulis	Passifloraceae	passionfruit
Prunus armeniaca	Rosaceae	apricot
Prunus armeniaca X salicina	Rosaceae	apricot-plum hybrid
Pulicaria arabica	Asteraceae	-
Pulicaria undulata	Asteraceae	-
Tamarindus indica	Fabaceae	tamarind

Conclusion: Pseudoionone is not a rare compound in plant biochemistry. Based on reports there seems to be a strong preponderance of dicotyledoneans, with many reports from nightshades (Solanaceae) and leguminous plants (Fabaceae).

Reliability: (4) not assignable
Various primary and secondary sources. Reliability 4.

21-NOV-2003 (2) (11) (17) (21) (38) (39) (48) (55) (63) (64) (65) (71) (74) (82) (84) (94) (100)

Memo: Natural formation of pseudoionone in tea leaves through fermentation and photo-oxidation

Result: Kawakami and Shibamoto (1991) analysed the volatile constituents of "toyama kurocha" piled tea, which is a special fermented tea used in the Japanese tea ceremony. For the preparation of "toyama kurocha", tea leaves (Camellia sinensis, Theaceae) are picked, steamed for 30 seconds, rolled for 10 minutes, fermented in a wooden frame on straw mats for 20-25 days and finally dried under the sun during 2-3 days. The mold Aspergillus niger is the predominant microbe in "toyama kurocha" manufacturing. Kawakami and Shibamoto extracted and analysed by GC/FID and GC/MS the volatile constituents of the various stages including samples of "toyama kurocha" stored for one year. They found no pseudoionone in the steamed fresh leaves, but 0.3 mg/100 g (dry weight) after fermentation, 0.5 mg/100 g dw after sun-drying and 0.2 mg/100 g dw after one year's storage. The authors note that the "aroma constituents of "toyama kurocha" were composed of many degradation prducts produced by microbial fermentation, photo-oxidation and auto-oxidation".

Conclusion: The formation of pseudoionone in Japanese fermented tea "toyama kurocha" evidently takes place during the microbial fermentation of steamed tea leaves and during sun-drying of these fermented leaves, as there was no pseudoionone detected in fresh tea leaves and as the concentration nearly doubled during the sun-drying process. During storage of "toyama kurocha" over one year, more than half of the pseudoionone degraded to undetermined metabolites. This is taken as evidence that pseudoionone may be formed from undetermined precursors both through microbial (Aspergillus) metabolism and through photo-oxidation; on the other hand, even in heavily dried product (only 10-13% water in "toyama kurocha"), pseudoionone will degrade over time.

Reliability: (2) valid with restrictions
Detailed methods and analytical procedures, concise reporting, reliability 2.

25-JUN-2003 (56)

Memo: Natural formation in plants or plant extracts through degradation or metabolism of higher terpenes

Result: In a GC-MS analytical determination of the constituents of a volatile oil steam-extracted from the plant Iochroma

gesnerioides (Solanaceae), the authors note the following: "C13, aber auch C8- und C18-Verbindungen sind ebenfalls vorhanden; es könnte sich um Abbauprodukte von höheren Terpenen handeln. [...] 6-Methyl-5-hepten-2-on, Geranylaceton und Farnesylaceton unterscheiden sich nur durch eine Isopreneinheit; sie sind vermutlich vom Lycopen abgeleitet. Die Verbindungen 32, 60 und 61 stellen verschiedene Oxydationsgrade dieser Ketone dar." (C13, but also C8 and C18 compounds are also present; they could represent degradation products of higher terpenes. [...] 6-Methyl-5-hepten-2-one, geranyl acetone and farnesyl acetone differ only by one isoprene unit; they are presumably derived from lycopene. The compounds 32, 60 and 61 [=pseudoionone] represent different degrees of oxydation of these ketones.)

Conclusion: Pseudoionone was identified in steam extracts of the plant Iochroma gesnerioides (Solanaceae). The authors assume pseudoionone, among other ketone compounds, to derive from degradation of the tetraterpene carotenoid lycopene.

04-JUN-2003 (2)

Memo: Formation of pseudoionone through degradation of plant carotenoids

Method: Modifications of flavour may occur during processing or storage of vegetable products, notably through the action of heat, oxygen or light on carotenoid pigments. To follow the degradation pathways and identify degradation products, 50 mg of beta-carotene or 15 mg of lycopene were suspended by sonication in 100 ml distilled water saturated with either oxygen or air in Kjeldal flasks; these were sealed and heated to 97±2 °C in an oil bath during 3 hours.
The volatile compounds produced by heat- and oxygen-induced degradation were isolated by dichloromethane extraction after elimination of undissolved products by filtration.
Identification of the extracted products was by gas chromatography (glass capillary column 40X0.4 mm inner diameter, Carbowax 20M operated at 50 °C during 10 min and then programmed to rise at 4 °C/min up to 170 °C). Mass spectrometry was also used for identification of compounds.

Result: Oxidative degradation of lycopene in air-saturated test solution resulted in the formation of pseudoionone (and geranial) through C10-C11 cleavage of all-trans-lycopene. It was made likely by following kinetic curves of production that the further product 6-methyl-3,5-heptadien-2-one (CAS 1604-28-0) was derived from pseudoionone in a second oxidative process.
In contrast, in oxygen-saturated test solution no pseudoionone was detected but 2-methyl-2-hepten-6-one through C6-C7 cleavage of lycopene. Oxidative degradation of beta-carotene did not lead to pseudoionone.

Conclusion: Pseudoionone may be formed from lycopene through oxidation during storage, at elevated temperatures and in simultaneous contact with air. This pseudoionone may be further oxidised in the process.
There is no evidence for pseudoionone formation through oxidation of beta-carotene.

Reliability: (2) valid with restrictions
Detailed publications with method and techniques, reliability judged to be 2.

24-JUN-2003 (53) (54)

Memo: Differential formation of pseudoionone depending on plant treatment

Method: In tobacco (Nicotiana tabacum) plants, removing the whole developing apical inflorescence (the flower bud) through so-called "topping" and/or removing the axillary bud or shoots (so-called "suckers") or controlling/reducing the sucker development through application of chemicals has an effect on the contents of nicotine and flavour compounds in the tobacco leaves. The relative effects of the following topping and sucker control actions were compared:
1) plants not topped and not suckered (controls); 2) plants topped but not suckered; 3) plants topped and suckers removed when 30 cm long; 4) plants topped and suckers removed when 20 cm long; 5) plants topped and suckers removed when 10 cm long; 6) plants topped and suckers removed before 1 cm long; 7) plants topped and suckers controlled chemically.
Leaves were harvested when considered ripe and cured (dried) in a bulk curing barn. After curing, 25 leaves per treatment were taken at random, weighed, the midribs removed and the lamina (leaf spreads) dried in a force-draft oven at 55 °C for 12 h and ground in a Wiley mill to pass a 1-mm mesh screen. This process was performed twice for each treatment. Subsamples were then prepared proportionally to earlier determined weights.
From these subsamples, total alkaloids (nicotine) and per cent reducing sugars were determined with an autanalyser according to Harvey et al. (Tobacco Sci 13: 13ff, 1969). Samples for gas chromatography were prepared by steam distillation of a 10-g subsample after Lloyd et al. (Tobacco Sci 20: 40ff, 1976). GC apparatus and conditions are described in detail.

Result: Regarding pseudoionone content, treament 7 (topping plus chemical sucker control) yielded the lowest content of 2.77 mg/g cured leaf. In contrast, treatment 6 (topping plus sucker removal before 1 cm long) yielded the highest pseudoionone content of 4.17 mg/g cured leaf.

Conclusion: Treatment of growing tobacco plants, specifically topping (removal of developing apical inflorescence) and sucker control (mechanical removal at different size or chemical suppression of axillary shoots) changes the biochemical content of tobacco leaves, in the case of pseudoionone either of pseudoionone itself or of the biochemical precursors.

Reliability: (2) valid with restrictions
Detailed paper with full methods and techniques, reliability 2.

25-JUN-2003 (105)

Memo: Occurrence in cured tobacco leaves and in tobacco smoke

Result: Pseudoionone has been shown to occur in tobacco plants, in cured (dried) tobacco leaves and in tobacco smoke. The occurrence of substances in cured tobacco leaves that do not or only to a lesser degree occur in fresh leaves is mainly ascribed to degradation of carotenoids, terpenoids and related substances. Because of the latter occurrence it has been investigated for various cytotoxic properties, see chapter 5.9, Specific Investigations.

Reliability: (4) not assignable

25-JUN-2003 (35) (64) (77) (79) (98)

1.12 Last Literature Search

Type of Search: External
Chapters covered: 3, 4, 5
Date of Search: 06-JUN-2003

Remark: Internet and SciFinder search.
06-JUN-2003 (87)

1.13 Reviews

Memo: RTECS

Result: Pseudoionone is listed in RTECS with identifiers including CAS no., synonyms, a dermal rabbit and an oral rat lethal dose and a reference to the TSCA status in the USA.
Reliability: (4) not assignable
Secondary source, reliability 4.
03-JAN-2003 (81)

Memo: Fragrance Raw Materials Monograph

Result: A two-page monograph with substance identifiers, occurrence, locations for chromatograms, regulatory status, biological data (acute toxicity, irritation, sensitisation, mutagenicity, teratogenicity, cytotoxicity) and references.
Reliability: (4) not assignable
Secondary source, reliability 4.
22-JAN-2003 (34)

2.1 Melting Point

Value: = -75 degree C

Method: other: dry ice/alcohol thermometer method.
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Test substance: Pseudoionone according to specifications, ie, cis/trans mixture, CAS 141-10-6.
Reliability: (2) valid with restrictions
Internal database, data at least 30 years old, acquired by company-internal physico-chemical properties laboratory. No information on method used is available, but data from this database are used and trusted within the company.
Flag: Critical study for SIDS endpoint
16-AUG-2004 (29)

2.2 Boiling Point

Value: = 265.4 degree C

Method: other: no data
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Test substance: Pseudoionone according to specifications, ie, cis/trans mixture, CAS 141-10-6.
Reliability: (2) valid with restrictions
Internal database, data at least 30 years old, acquired by company-internal physico-chemical properties laboratory. No information on method used is available, but data from this database are used and trusted within the company.
Flag: Critical study for SIDS endpoint
15-JAN-2004 (29)

Value: = 263.2 degree C

Method: other: no data
GLP: no
Test substance: other TS

Test substance: cis-Pseudoionone, CAS 33073-35-7.
Reliability: (2) valid with restrictions
Internal database, data at least 30 years old, acquired by company-internal physico-chemical properties laboratory. No information on method used is available, but data from this database are used and trusted within the company.
15-JAN-2004 (29)

Value: = 271 degree C

Method: other: no data
GLP: no
Test substance: other TS

Test substance: trans-Pseudoionone, CAS 3796-54-1.
Reliability: (2) valid with restrictions

Internal database, data at least 30 years old, acquired by company-internal physico-chemical properties laboratory. No information on method used is available, but data from this database are used and trusted within the company.

15-JAN-2004 (29)

Value: = 235 degree C

Method: other: no data
Year: 2000
GLP: no data
Test substance: no data

Result: In a paper on the development of a quantitative structure-property relationship for the boiling point at normal pressure of small organic molecules, pseudoionone is listed to have an experimental boiling point of 235 °C. The experimental dataset stems from 5 different cited references, however, the single values are not referenced.
Reliability: (4) not assignable
Only secondary source, primary source not identifiable, hence reliabilty 4.

05-JUN-2003 (92)

2.3 Density

Type: density
Value: = .8951 g/cm³ at 20 degree C

Method: other: double-capillary pycnometer
Year: 1988
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: The measurement of liquid density was carried out in multiplicate by using a double-capillary pycnometer calibrated with double-distilled de-gassed water. The method error as determined by measurements of acetone and of methanol was 0.02%.
Result:

Temperature, °C	Density, g/cm3
20.0	0.8951
30.0	0.8875
40.0	0.8797
50.0	0.8721
60.0	0.8644
70.0	0.8573

Test substance: Commercial pseudoionone was purified by drying over Na2SO4, MgSO4, K2CO3 and CaCl2, rectified through columns with efficiency equal to 50 theoretical column trays and multistage-fractionally-distilled at residual pressure varying from 6.7 to 67 Pa.
The output content of the product was determined by area normalisation of gas-liquid chromatography curves. The purified pseudoionone used for the present determination was determined to have a purity of 98.50 mol-%.
Reliability: (2) valid with restrictions
Although this was not a study under GLP or similar conditions, both preparation and careful purification of samples are described, experimental methods are briefly but concisely

presented, these methods are validated against literature data and the calibration results are presented. Experimental data are listed in full. Based on these ample descriptions and internal quality control data, a reliability of 2 is assigned.
Flag: Critical study for SIDS endpoint
09-JAN-2003 (4)

Type: density
Value: = .8952 g/cm³ at 20 degree C

Method: other: no data
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Reliability: (2) valid with restrictions
Internal database, data at least 30 years old, acquired by company-internal physico-chemical properties laboratory. No information on method used is available, but data from this database are used and trusted within the company.
15-JAN-2004 (29)

Type: density
Value: = .6864 g/cm³ at 265.4 degree C

Method: other: no data
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Reliability: (2) valid with restrictions
Internal database, data at least 30 years old, acquired by company-internal physico-chemical properties laboratory. No information on method used is available, but data from this database are used and trusted within the company.
15-JAN-2004 (29)

2.3.1 Granulometry

2.4 Vapour Pressure

Value: = .001741 hPa at 20 degree C

Method: other (measured): no data
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Reliability: (2) valid with restrictions
Internal database, data at least 30 years old, acquired by company-internal physico-chemical properties laboratory. No information on method used is available, but data from this database are used and trusted within the company.
Flag: Critical study for SIDS endpoint
10-JAN-2006 (29)

Value: = 2.8 hPa at 109.4 degree C

Year: 1988
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: Vapour pressure was measured by a static method [cited in the paper as Baglay et al (1984): Khim.-Farm. Zh. 18: 1013 ff, in Russian] with a glass membrane as a null manometer. Nonvolatile compounds were introduced into the membrane camera immediately. The tensimeter was embedded in an oil or LiCl-water-solution thermostat, which allows the measurement of the temperature using a mercury thermometer with an error of ±0.1 K. Pressure was measured with a cup mercury manometer with an accuracy of ±13.3 Pa.

Result: Experimental vapour pressures are given for the range of 109.41-184.3 °C (in the original 382.56-457.45 K):

Temperature, K	°C	Vapour pressure, hPa
382.56	109.41	2.8
387.95	114.8	3.3
389.55	116.4	3.6
398.85	125.7	6.4
406.95	133.8	9.6
407.75	134.6	10.4
417.95	144.8	16.7
418.35	145.2	17.2
428.85	155.7	26.3
430.25	157.1	28.5
433.65	160.5	35.2
435.45	162.3	42.0
445.65	172.5	51.2
448.85	175.7	58.8
452.25	179.1	66.2
457.45	184.3	81.7

Extrapolation of the value at 382.56 K (109.41 °C) to 293.15 K results in a vapour pressure of 0.00183 hPa at 20 °C.

Test substance: Commercial pseudoionone was purified by drying over Na2SO4, MgSO4, K2CO3 and CaCl2, rectified through columns with efficiency equal to 50 theoretical column trays and multistage-fractionally-distilled at residual pressure varying from 6.7 to 67 Pa.
The output content of the product was determined by area normalisation of gas-liquid chromatography curves. The purified pseudoionone used for the present determination was determined to have a purity of 98.50 mol-%.

Reliability: (2) valid with restrictions
Although this was not a study under GLP or similar conditions, both preparation and careful purification of samples are described, experimental methods are briefly but concisely presented, these methods are validated against literature data and the calibration results are presented. Experimental data are listed in full. Based on these ample descriptions and internal quality control data, a reliability of 2 is assigned.

10-JAN-2006 (4)

2.5 Partition Coefficient

Partition Coeff.: octanol-water
log Pow: = 3.9 - 4.1 at 25 degree C
Method: Directive 84/449/EEC, A.8 "Partition coefficient"

Year: 1989
GLP: no data

Method: The HPLC method was used for determination of the n-octanol/water partition coefficient of pseudoionone.
HPLC conditions

HPLC	Varian 5000
Column	Lichrospher 100 C18 (Merck, Germany)
particle size	5 µm
inner diameter	4 mm
length	100 mm
Column temperature	25±2 °C
Injector	Rheodyne 7125; 10-µl loop
Concentration of standards	approximately 100 ppm m/m in methanol
Concentration of pseudoionone	0.1% in methanol
UV detector	PE LC 75
Wavelength	210 nm
Eluent	25% water/75% methanol v/v
Flux	1.5 ml/min
Pressure	110 bar
Recorder	Servor analogue recorder 220

Standards according to EEC directive 79/831:

Chlorobenzene	Aldrich Germany	logPow = 2.8
Benzophenone	Aldrich Germany	logPow = 3.2
Phenylbenzoate	Aldrich Germany	logPow = 3.6
Diphenyl ether	Aldrich Germany	logPow = 4.2
n-Butyl benzene	Aldrich Germany	logPow = 4.5
Dibenzyl	Aldrich Germany	logPow = 4.8
Triphenylamine	Aldrich Germany	logPow = 5.7
DDT	Polyscience Germany	logPow = 6.2

The test substance and the standards were injected in triplicate. logPow was interpolated using the log retention time/logPow regression line given by the standards and using the mean retention times for the two pseudoionone isomers.

Result: n-Octanol/water partition coefficient for pseudoionone:

	average logk'	logPow
1st isomer	0.670	3.9
2nd isomer	0.719	4.1

Conclusion: The logPow for pseudoionone is 3.9 and 4.1 for the two isomers.
Reliability: (2) valid with restrictions
While there is no information on GLP or not, an official EC guideline was adopted, the test report is short but concise, giving full HPLC conditions, identity and logPow of the standards, average retention values for test substance and standards and the computed logPow. Further, the test was performed in a professional analytical laboratory. Reliability is set at 2.
Flag: Critical study for SIDS endpoint
06-JAN-2003 (15)

Partition Coeff.: octanol-water
log Pow: 3.54 - 4.57

Method: other (calculated)
Year: 2003

GLP: no

Method: The SMILES code for pseudoionone was entered into two online and one downloadable programmes.

Result:

Computed logPow	QSPR Program	Source
3.54	XLOGP	VCC-Lab
3.58	CLOGP	VCC-Lab
3.83	ACD Solaris V4.67	SciFinder
3.85	SPARC	SPARC
4.43	KOWWIN	EPISUITE v.3.10
4.48	IA logP	VCC-Lab
4.57	ALOGPs	VCC-Lab

4.04 average value		

Test substance: Pseudoionone, entered as the SMILES notation.
Conclusion: The average of 7 computer-estimated n-octanol/water partition coefficients is 4.04, which agrees very well with the experimental value of 3.9-4.1.
Reliability: (2) valid with restrictions
QSPR computer programs, commonly accepted, reliability 2.

10-JAN-2006 (23) (90) (104)

Partition Coeff.: water - air

Method: other (calculated)
Year: 2003
GLP: no

Result:

Henry's Constant, KH, atm*m3/mol	Estimation Program	Source
3.47E-4	HENRYWIN, bond est.	EPISuite v.3.10
1.34E-5	HENRYWIN, group est.	EPISuite v.3.10
1.22E-5	SPARC	SPARC
5.22E-6	HENRYWIN, VP/WSol est.	EPISuite v.3.10
3.40E-6*	EUSES	EUSES v.1.0
---		

*KH given in EUSES as 0.345 Pa*m3/mol, conversion factor Pa->atm = 9.86923*10E-6.

Test substance: Pseudoionone, entered as the SMILES notation.
Conclusion: With calculated Henry's Law Constants between 3.4E-6 and 3.5E-4, pseudoionone is predicted to be of moderate to low volatility from water.
Reliability: (2) valid with restrictions
QSPR computer programs, commonly accepted, reliability 2.

10-JAN-2006 (23) (25) (90)

Partition Coeff.: soil-water

Method: other (calculated)
Year: 2003
GLP: no

Result:

Koc	logKoc	QSAR program	Source
696	2.84	PCKoc v1.66	EPISuite v.3.10
2880	3.46	ACD Solaris V4.67	SciFinder

Test substance: Pseudoionone, entered as the SMILES notation.
Conclusion: With a QSAR-predicted Koc between 696 and 2880, pseudoionone is expected to adsorb moderately to organic carbon and,

conversely, to be relatively mobile in soil.
Reliability: (2) valid with restrictions
QSAR calculations, commonly accepted, reliability 2.
10-JAN-2006 (23) (87)

2.6.1 Solubility in different media

Solubility in: Water
Value: = 97 mg/l at 25 degree C

Method: other: no data
Year: 1989
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Reliability: (2) valid with restrictions
While details are lacking, the study was performed in a laboratory of a big competent chemical-pharmaceutical company that is a co-producer and co-sponsor of this report.
Flag: Critical study for SIDS endpoint
15-JAN-2004 (6)

Solubility in: Water
Value: = .1 g/l

Method: other: no data
GLP: no data

Remark: Secondary source corroborating the water solubility.
Test substance: Test substance described as "2-pseudoionone", no other information (secondary source).
Reliability: (4) not assignable
Secondary source, reliability 4.
03-JUN-2003 (91)

2.6.2 Surface Tension

Test type: other: capillary method
Value: = 32.3 mN/m at 20 degree C
Concentration: 95 other: mol-%

Year: 1988
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: The liquid-gas surface tension was measured by the capillary method as described by Baglay et al. [1984: Khim.-Farm. Zh. 18: 1013 ff, in Russian] and Adamson [1979: Physical chemistry of surfaces. Mir, Moscow; in Russian]. The level of liquid in the capillary was determined by a V-630 type cathetometer with an accuracy of ±5x10E-6 m. The relative error of the surface tension experimental data determined from water, toluene and n-octane was <0.5%.
Remark: Based on the OECD Test Guideline 115, substance with a surface tension below 60 mN/m at 20 °C should be regarded as surface-active. With a surface tension of 32.3 mN/m, pseudoionone should therefore be regarded as surface-active.
Result: Temperature, °C Surface tension, mN/m

20.0	32.30
30.0	31.61
40.0	30.72
50.0	29.88
60.0	28.87
70.0	27.95

Test substance: Commercial pseudoionone was purified by drying over Na2SO4, MgSO4, K2CO3 and CaCl2, rectified through columns with efficiency equal to 50 theoretical column trays and multistage-fractionally-distilled at residual pressure varying from 6.7 to 67 Pa.
The output content of the product was determined by area normalisation of gas-liquid chromatography curves. The purified pseudoionone used for the present determination was determined to have a purity of 98.50 mol-%.

Reliability: (2) valid with restrictions
Although this was not a study under GLP or similar conditions, both preparation and careful purification of samples are described, experimental methods are briefly but concisely presented, these methods are validated against literature data and the calibration results are presented. Experimental data are listed in full. Based on these ample descriptions and internal quality control data, a reliability of 2 is assigned.

Flag: Critical study for SIDS endpoint

09-JAN-2003 (4)

Value: = 27.27 mN/m at 20 degree C

Method: other: no data
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Reliability: (2) valid with restrictions
Internal database, data at least 30 years old, acquired by company-internal physico-chemical properties laboratory. No information on method used is available, but data from this database are used and trusted within the company.

15-JAN-2004 (29)

Value: = 9.83 mN/m at 265.4 degree C

Method: other: no data
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Reliability: (2) valid with restrictions
Internal database, data at least 30 years old, acquired by company-internal physico-chemical properties laboratory. No information on method used is available, but data from this database are used and trusted within the company.

15-JAN-2004 (29)

2.7 Flash Point

Value: = 97 degree C

Method: other: no data
GLP: no

Test substance: as prescribed by 1.1 - 1.4

Reliability: (2) valid with restrictions
Internal database, data at least 30 years old, acquired by company-internal physico-chemical properties laboratory. No information on method used is available, but data from this database are used and trusted within the company.
Flag: Critical study for SIDS endpoint
15-JAN-2004 (29)

2.8 Auto Flammability

Value: = 260 degree C at 1013 hPa

Method: other: no data
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Reliability: (2) valid with restrictions
Internal database, data at least 30 years old, acquired by company-internal physico-chemical properties laboratory. No information on method used is available, but data from this database are used and trusted within the company.
Flag: Critical study for SIDS endpoint
15-JAN-2004 (29)

Value:

Year: 1996
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: The mechanism and kinetics of autoxidation of a series of polyene compounds was investigated.
The test substances including purified pseudoionone were either tested dissolved in chlorobenzene at 45 °C or as a thin "solid" film on a support at room temperature. Preparation of the test substances and exposure forms followed procedures described by the same authors previously (Finkelshtein et al., Int J Chem Kinet 16: 513-524, 1984; Finkelshtein & Kozlov, Photochem Photobiol 30: 313-316, 1979).
Both forms of prepared test substances were exposed in a test chamber with an apparatus measuring the rate of oxygen absorption respectively the delivery of oxygen. The apparatus consisted of a reaction cell, differential contact mercury micromanometer and a hypodermic syringe. All these parts of the apparatus were thermostated by water jackets at 45 °C. The connecting capillary tubes were thoroughly insulated by foamed plastic. The piston of the syringe was geared to a synchronous servo motor and a linear potentiometer-recorder system. The imbalance of the pressure was corrected by moving the syringe piston by the servo motor switched on by contact micromanometer through an electronic relay. The displacement of the piston was converted into voltage changes by the potentiometer and the output signal was recorded by a strip-chart recorder. The sensitivity of the apparatus was about 10E-8 mol of O2 per 1 mm of recorder scale.
During the experiments, spectra of the substances and autoxidation products were recorded. The electronic spectra of

solutions and films were recorded on a Specord M40 instrument (Carl Zeiss, Jeny, Germany). The procedures for recording infrared spectra (transmission and attenuated total reflection were described in an earlier publication (Krasnokutskaya & Finkelshtein, J Mol Struct 349: 313-316, 1995).

Result: The authors describe mechanisms and kinetics of autoxidation of a series of polyene compounds using the numer of sites attacked by oxidation and the number of isomerised radicals produced in a given series of autoxidation products. Propagation and termination rate constants for the autoxidation were determined. The results "lead to the conclusion that polyenes with 'allylic' hydrogens (2 [= retinyl acetate] and 8 [= pseudoionone]) are more reactive than polyenes undergoing initial addition of peroxyls to the polyene chain (3-6 [all-E-methyl reinoate, retinal, C18-ketone, beta-ionylidene acetaldehyde] and 9 [ethyl sorbate])."

Test substance: Commercial technical pseudoionone (in the publication designated as psi-ionone) was vacuum-distilled to a purity of 99.7% according to GC analysis.

Conclusion: In a highly technical experiment, pseudoionone was identified as being more reactive respectively susceptible to autoxidation than a series of other polyene compounds. This confirms the hazard of autoxidation of pseudoionone.

Reliability: (2) valid with restrictions
Detailed technical publication with clear methods, description of results and derivation of mechanisms. Reliability 2.

05-JUN-2003 (31)

Value:

Method: other: incident report

GLP: no

Test substance: as prescribed by 1.1 - 1.4

Result: On 05-DEC-2002 a minor incident involving self-ignition of pseudoionone happened in the Teranol plant, Lalden, Switzerland.
Cleaning material humid or wet with pseudoionone had been deposited in a sink (room temperature). Subsequently, the time delay is not known, the material self-ignited, causing a minor fire. The fire was noted by a production worker, the summoned fire brigade rapidly extinguished the fire. There was no harm to people, no effects on the environment but minor business/financial damage caused by this incident.
Self-ignition of pseudoionone on material with a large surface is noted in the report as "a well-known phenomenon".
Corrective action stated was "proper disposal of [cleaning material] into the appropriate disposal boxes (self-extinguishing)".

Test substance: Technical pseudoionone from Teranol Lalden, corresponding to specifications.

Conclusion: On materials with a large material/air interface, such as cleaning materials/rags, pseudoionone may auto-ignite at room temperature.

Reliability: (2) valid with restrictions
Accident/Incident report from a Roche plant with detailed description of conditions and sequence of the incidence. Reliability set at 2.

Flag: Critical study for SIDS endpoint

06-JUN-2003 (26)

Value:

Method: other: incident report
Year: 1986
Test substance: other TS

Result: From a Roche 1975 incident report, Roche Vitamins Plant Sisseln, Switzerland:
A minor beta-ionone spill was taken up with rags. The soaked rags were put in a metal bucket and left outside of the production building. Within a short time, a few minutes, the rags were "burning brightly", having self-ignited.
Test substance: beta-Ionone, CAS 14901-07-6, closed-ring isomer of pseudoionone.
Conclusion: Based on experience with the chemically closely related beta-ionone, the possibility of auto-flammability of pseudoionone under special circumstances, such as large contact area with air in the case of soaked rags, should be considered.
Reliability: (4) not assignable
Conclusion by analogy, reasonable but unsubstantiated.
Reliability 4.
06-JUN-2003 (107)

2.9 Flammability

2.10 Explosive Properties

2.11 Oxidizing Properties

2.12 Dissociation Constant

Acid-base Const.: none

Method: other: QSAR-calculated
Year: 2003
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Result: According to the SPARC Online Calculator and a Roche-internal QSAR CpKa application, pseudoionone is not expected to dissociate at any environmentally relevant pH.
Reliability: (4) not assignable
19-JUN-2003 (80) (90)

2.13 Viscosity

Test type: other: dynamic, exact type unknown
Method: other: no data
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Result: Temperature, °C Viscosity, kg/(m*s)
20.0 0.00571
265.4 0.00000197
Reliability: (2) valid with restrictions
Internal database, data at least 30 years old, acquired by company-internal physico-chemical properties laboratory. No information on method used is available, but data from this database are used and trusted within the company.
15-JAN-2004 (29)

2.14 Additional Remarks

Memo: Refraction index

Method: no data
Result: refraction index (20 °C) = 1.5313
Test substance: Commercial pseudoionone was purified by drying over NaSO4, MgSO4, K2CO3 and CaCl2, rectified through columns with efficiency equal to 50 theoretical column trays and multistage-fractionally-distilled at residual pressure varying from 6.7 to 67 Pa.
The output content of the product was determined by area normalisation of gas-liquid chromatography curves. The purified pseudoionone used for the present determination was determined to have a purity of 98.50 mol-%.

Reliability: (4) not assignable
Basically reliable source but no method given for refraction index. Reliability 4.
09-JAN-2003 (4)

Memo: Stability during roasting

Method: Green, dried fermented leaves and roasted, fermented leaves of mate tea (Ilex paraguayensis, Aquifoliaceae) were steam-distilled and the distillates subsequently analysed by GC and GC-MS (full details in paper).
Result: The distillate from roasted fermented mate leaves showed a pseudoionone content that was approximately one-third (GC peak area) that of the distillate from dried green, fermented mate leaves.
Conclusion: Pseuodionone is not thermally stable, it degrades or possibly evaporates during roasting at unstated temperatures for an unstated time.
Reliability: (2) valid with restrictions
Full preparation and analytical details. Reliability 2.
14-JAN-2003 (55)

Memo: Volatility

Method: In a honeybee spatial (olfactory) repellence test, a circular disc coated with silica gel, outer diameter 3.2 cm and inner diameter 2.0 cm, was cut out of Eastman-Kodak No. 6060 silica gel. Such discs were immersed into known concentrations of candidate repellent test substances, "usually dissolved in 95% ethanol". The solvent was allowed to dissipate in a vented hood, the disc bearing the candidate repellent was placed over a feeder vial containing a 1:1 mixture of honey and water and the vial was capped with a cap with 1.5-mm holes to allow the

honeybees to feed.
In order to test the volatility characteristics of the respective test substances, samples of each repellent were dissolved in ethanol and adsorbed on tared discs coated with silica gel as above and placed in a hood at 27 °C, 35-45% RH at an airflow of 57 l/s.Following evaporation of the solvent, the discs were re-weighed at regular intervals over a period of 24 hours and the results plotted on logarithmic graph paper. The volatility half-life was determined from these graphs, probably by hand (not stated).

Result: The volatility half-life for (E)-pseudoionone is given as >24 hours, the upper limit of the test.

Test substance: (E)-Pseudoionone, source and purity not stated.

Reliability: (2) valid with restrictions
Detailed publication with clear (non-OECD) methods but only summary results, reliability 2.

03-JUN-2003 (3)

Memo: Stability during gas chromatography

Result: Abstract text:
The gas chromatographic behaviour of psi-Ionone (I), CAS 141-10-6 [=pseudoionone], and 6,10,14-trimethylpentadeca-3,5-dien-2-one (II), CAS 1604-32-6, intermediates in the synthesis of vitamins A and E, was studied on inert carriers, Chromatone NAW (0.20-0.25 mm) and Inerton AW-HMDS, CAS 98668-09-8 (0.16-0.29 mm) which were silanised. The degradation of I and II depended on the length of the column and the temperature. The degradation of the ketones was 43% and 36%, respectively, when Chromatone NAW was used at 160 °C, length 2.5 m and 1.28 m. It is recommended that acid-washed Chromatone NAW must not be used. Inerton AW-HMDS did not decompose the ketones.

Conclusion: Pseudoionone may decompose during gas chromatography, depending on the column carrier used and the temperature and time/column length applied.

14-JAN-2003 (30)

3.1.1 Photodegradation

Type: air

Remark: No experimental data have been located.
Result: The EPISuite QSAR model predicts the following atmospheric degradation half-lives for pseudoionone:
·OH-mediated 29.5 min (12-h day, 1.5*E+6 ·OH/cm3 air)
O3-mediated 12.2 min (7*E+11 mol O3/cm3 air)
·NO3 radicals may be important for degradation reactions.
Total estimated atmospheric half-life: 10.2 min
Conclusion: Based on QSAR modelling, pseudoionone is expected to be rapidly degraded in the atmosphere. It is not expected to be a persistent substance in air.
Reliability: (2) valid with restrictions
Computer model package approved, used and distributed by US EPA, reliability 2.
Flag: Critical study for SIDS endpoint
15-JAN-2004 (23)

3.1.2 Stability in Water

Type: abiotic

Method: other: reasoning based on chemical structure and QSAR expert system
Year: 2004
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: Based on the chemical structure, in particular on the absence of hydrolysable bonds, pseudoionone is expected to be stable in water.
Reliability: (2) valid with restrictions
Sound scientific reasoning and using a QSAR expert system, but no GLP, reliability 2.
Flag: Critical study for SIDS endpoint
10-JAN-2006 (90)

3.1.3 Stability in Soil

3.2.1 Monitoring Data (Environment)

3.2.2 Field Studies

3.3.1 Transport between Environmental Compartments

3.3.2 Distribution

Media: other: static distribution in air - biota - sediment(s) - soil - water

Method: Calculation according Mackay, Level I
Year: 2003

Result:

Compartment	Level I amount, %
Air	0.699
Water	9.87
Soil	87.4
Sediment	1.94
Suspended particles	0.0607
Fish	0.00494

Conclusion: In a static fugacity-driven distribution model without advection or reaction, pseudoionone is expected to distribute mainly to soil (approximately 87%) and water (10%), with sediment (2%), air (0.7%), suspended particles (0.07%) and fish (0.005%) being serially less important compartments.
Reliability: (2) valid with restrictions
Widely used and accepted computer distribution model, reliability 2.
Flag: Critical study for SIDS endpoint
15-JAN-2004 (24)

Media: other: dynamic distribution in air - biota - sediment(s) - soil - water
Method: Calculation according Mackay, Level III
Year: 2003

Result: Dynamic distribution, Level III amount, %

Emissions, kg/h, to	air	water	soil	sediment
	1	0	0	0
Compartment				
Air	70.4			
Water	9.1			
Soil	15.5			
Sediment	4.98			
Residence time, h	0.343			

	0	1	0	0
Compartment				
Air		0.000542		
Water		64.6		
Soil		0.000119		
Sediment		35.3		
Residence time, h		353		

	0	0	1	0
Compartment				
Air			0.0000159	
Water			0.0601	
Soil			99.9	
Sediment			0.0329	
Residence time, h			300	

	0	0	0	1
Compartment				
Air				0.00000847
Water				1.01
Soil				0.00000186
Sediment				99.0
Residence time, h				15641

Assumed realistic emissions only to air and water:

	1	1	0	0
Air	0.0689			
Water	64.2			
Soil	0.0152			
Sediment	35.3			
Suspended particles	0.395			
Fish	0.0321			
Residence time, h	177			

Model conditions
Half-lives from EPISuite v3.10, using primary degradation rates: air and aerosols = 0.168 h; water, suspended solids, soil and fish = 208 h; sediment = 10E11 h (negligible, based on the anaerobic biodegradation test).

Conclusion: The Level III dynamic distribution model highlights the importance of the emission pathway, with huge resulting differences in steady-state distributions and average residence times. For realistic emissions, only to air and water, from a closed production system with exceptional breaching or from closed or nearly closed further processing systems, the main distribution is expected to water (64.2%) and secondarily to sediment (35.5%), while suspended particles (0.4%), air (0.07%) and fish 0.03%) are comparatively unimportant.

Reliability: (2) valid with restrictions
Widely used and accepted computer distribution model, reliability 2.

Flag: Critical study for SIDS endpoint

15-JAN-2004 (24)

3.4 Mode of Degradation in Actual Use

3.5 Biodegradation

Type: aerobic
Inoculum: other: activated sludge from a laboratory wastewater treatment plant using municipal sludge
Concentration: 45 mg/l related to Test substance
Contact time: 28 day(s)
Degradation: = 62 % after 27 day(s)
Result: other: well biodegradable but missed ready biodegradability because of 10-day criterion
Kinetic:

6 day(s)	= 0 %
7 day(s)	= 5 %
8 day(s)	= 32 %
17 day(s)	= 50 %
25 day(s)	= 61 %

Control Subst.: Aniline
Deg. product: not measured

Method: OECD Guide-line 301 F "Ready Biodegradability: Manometric Respirometry Test"
Year: 1988
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: A manometric biodegradability test was performed in the BASF

Laboratory of Emission Control in Ludgwigshafen, Germany, in 1988, according to OECD Guideline 301F and ISO Guideline 9408. A concentration of 45 mg pseudoionone/l medium was incubated with 30 mg (dry weight) activated sludge/l medium in a closed respirometer in duplicate, in parallel a reference substance flask (aniline, 100 mg/l) was run. The biochemical oxygen demand was registered electronically and related to the theoretical oxygen demand for complete oxidation of the test substance. The test started on 29-Jan-1988 and ran for 28 days.

Result: In a standard respirometric test comparing biochemical oxygen demand to theoretical oxygen demand (BOD/ThOD), pseudoionone reached 62% biodegradation in 28 days.
As evidenced by the data table and the degradation graph, pseudoionone exerted an initial inhibition of the activated sludge until day 7, when biodegradation finally started. Subsequently, degradation took a leap to over 30% within one single day, after which the curve started to flatten and degradation proceeded nearly linearly, reaching 62% on day 27. The mark of 60% was not reached within 10 days from day 8, however.

Test substance: Pseudoionone from BASF AG, batch and purity not stated.

Conclusion: Pseudoionone was shown to be biodegradable in a standard ready test over 28 days, however, as the 10-day-window criterion was not met, it cannot be said to be readily biodegradable. At the start there was a delay of 6 days until biodegradation took off, suggesting the need for an adaptation phase for the activated sludge.

Reliability: (2) valid with restrictions

Reprint of test report based on original laboratory data, including details as to test substance concentration, reference substance concentration, data table and degradation graph, from a professional industry biodegradation laboratory, following international guidelines. Reliability 2.

Flag: Critical study for SIDS endpoint

22-JAN-2003 (75)

Type: anaerobic
Inoculum: anaerobic sludge
Concentration: 122 mg/l related to Test substance
Contact time: 93 day(s)
Degradation: = 0 % after 93 day(s)
Result: under test conditions no biodegradation observed
93 day(s) = 0 %
Control Subst.: Diethylene glycol
Kinetic: 41 day(s) = 82 %
Deg. product: not measured

Method: other: ISO 11734
Year: 2002
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: An Ultimate Anaerobic Degradation test was performed according to ISO Guideline 11734.
Briefly, three replicate pseudoionone flasks, three incolum blank flasks and two diethylene glycol positive control flasks were run in parallel. The flasks were 1222-ml glass bottles closed with hermetically sealing butyl rubber

stoppers with ports and a manometer attached. The flasks contained a test solution volume of 800 ml, made up of digested sludge from the digester of the biological step of the municipal sewage works ARA Werdhölzli in Zürich, Switzerland, at 2 g/l (dry matter) in the final mixture, with defined mineral salts according to ISO 11734 (details in report) in de-aerated water and either pseudoionone at a loading concentration of 99.3 mg total organic carbon (TOC)/l (= 122 mg pseudoionone/l) as the only organic carbon source for the test flasks; of 45.6 mg TOC/l (= 100.9 mg diethylene glycol/l) for the control flasks; or nothing else for the inoculum controls. The flasks were filled with the de-aerated medium and substances as above, the headspace was filled with nitrogen gas and stoppered.
Test flasks were incubated at 35±2 °C in the dark and agitated once a day except on weekends. Determination of anaerobic biodegradation was made by precisely measuring the pressure in the headspace using a MP340A measuring device by EIRELEC Ltd, bleeding of the excess biogas volume and determining the inorganic carbon (IC) in the excess biogas with a Shimadzu 5050 TOC-Analyzer. Based on IC concentration, headspace volume and pressure, the amount of IC produced since the last measurement can be calculated and summed up. The IC produced by the inoculum blank serves as a baseline and is subtracted from the test and control values. IC divided by TOC gives the degradation at a time point. At the end of the test, the remaining IC in the aqueous phase is also determined and added to the headspace IC to give the final degradation.

Result: Anaerobic degradation was followed over 93 days.

Degradation kinetics:	day	%degradation (baseline = inoculum blank)	
Pseudoionone	0	0	
	6	-20	(after subtraction of blank IC)
	34	-38	
	55	-42	
	93	-49	(headspace IC only)
	93	-74	(total IC including liquid)
Diethylene glycol (positive control)	0	0	
	3	11	
	13	66	
	41	82	(plateau)
	55	83	

The negative degradation from the beginning shows a clear initial inhibition of the (non-adapted) digested sludge by pseudoionone. This inhibition reaches a (negative) plateau around day 55, but there is no indication of degradation. The positive control showed rapid degradation of diethylene glycol, reaching a plateau of approximately 82% degradation on day 41.

Test substance: Pseudoionone from Teranol, Lalden, Lot no. UU02033826, purity 96.1% (area, GC).

Conclusion: Pseudoionone was not anaerobically biodegradable in a prolonged standard test. Moreover, at a loading concentration of 122 mg/l it was consistently toxic respectively inhibitory to the anaerobic sludge bacteria.

Reliability: (2) valid with restrictions
While BMG Engineering Ltd are not GLP-certified, they adhere to quality assurance system SN EN 45001. The test report is concise and detailed, with all single basic data, measurements, calculations and graphs given, hence reliability

was set 2.
Flag: Critical study for SIDS endpoint
11-JUN-2003 (45)

Type: aerobic
Inoculum: other fungi: Aphanocladium album (Hyphomycetes) and Rhodotorula mucilaginosa (Basidiomycetes)
Concentration: 120 mg/l related to Test substance
Contact time: 14 day(s)
Degradation: = 100 % after 14 day(s)
Result: other: 100% primary biodegradation in 14 days, no information on further degradation
Deg. product: yes
116048-77-2 5,9-undecadien-2-ol, 6,10-dimethyl, (R)-
50373-44-9 5,9-undecadien-2-ol, 6,10-dimethyl, (S)-
689-67-8 211-711-2 6,10-dimethylundeca-5,9-dien-2-one

Year: 1987
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: The ability of two fungi, the insect parasite Aphanocladium album (Hyphomycetes) and the soil yeast Rhodotorula mucilaginosa (Basidiomycetes), to degrade respectively transform some juvenoid-type compounds was investigated. The Aphanocladium strain was originally isolated from mealworms, Tenebrio molitor, and the Rhodotorula strain from soil; both strains were obtained from the Culture Collection of the Laboratory of Biology and Botany of the Medical Academy of Wroclaw, Poland.
The transformations were carried out using a submerged culture method. The strains were cultivated in 2-litre flasks containing 1 litre of a maltose nutrient at 27 °C each, with constant shaking. After 3 days of growth, 120 mg of the respective transformation substrates including pseudoionone was added to 1 litre of culture. The transformation was carried out for 14 days and then the products were extracted with chloroform. The crude product mixture was separated on columns filled with silica gel. Hexane-ethyl ether mixtures were used as an eluent.
For product identification, spectral analyses were made on the following instruments:
IR: UR-20, Zeiss (films);
1H-NMR: Tesla 100 MHz and Varian 100 MHz, standard TMS;
Optical rotation: Polamat A, Zeiss, standard CHCl3 = 1;
GLC: N504 Elwro, Wroclaw, Poland (FID, 2-m columns filled with 10% Carbowax 20 M on Chromosorb W AW DMCS, 80-100 mesh, temperature 150 °C, carrier gas nitrogen, flow 50 ml/min.
Result: In experiment 4, with pseudoionone as the starting substrate, the transformation of pseudoionone with Aphanocladium resulted in 45 mg/l of isolated (+)-6,10-dimethyl-5,9-undecadien-2-ol [CAS 50373-44-9, analytical parameters given], while the transformation with Rhodotorula resulted in 30 mg/l of isolated 6,10-dimethyl-5,9-undecadien-2-one [689-67-8, parameters given] and 8 mg/l of isolated (-)-6,10-dimethyl-5,9-undecadien-2-ol [CAS 116048-77-2, parameters given]. No unreacted ketone was found among isolated products after 14 days.
The authors note that due to relatively low chloroform extractability and the relatively high volatility, the amounts

isolated are not to be regarded as absolute but only related to the chloroform extract.

Test substance: "Pseudoionone and [...] were obtained from racemic citronellol, as described elsewhere [Galera E, Zabza A (1977): Insect growth regulators. II. C-15 derivatives of ethyl-6,7-dihydrofarnesoate. Bull Acad Polon Sci, Ser Sci Chim 25: 615-625]." Full spectral details for pseudoionone are given but no information on purity.

Conclusion: Two fungi, Aphanocladium and Rhodotorula, both tranformed pseudoionone completely within 14 days, no original substance was left. Both fungi acted by hydrogenation of the double bond at C3 with subsequent reduction of the carbonyl group.
Due toincomplete recovery caused by low chloroform extractability and relativel high volatility of products, no other products could be identified nor fully quantified. It was shown, however, that the two fungi differed in the optical rotation and relative amount of products.

Reliability: (2) valid with restrictions
In spite of lack of information regarding the purity of the test substance pseudoionone, the methods and particularly the identification of both pseudoionone and the three metabolites are presented in detail. Reliability judged 2.

10-JAN-2006 (22)

Type: aerobic
Inoculum: other bacteria: "bacteria in sea water"

Method: other: no data
Year: 1982
GLP: no data
Test substance: no data

Remark: Kuraray declined to give further information on this patent.
Result: By searching for the CAS number 141-10-6, a location of a Japanese patent was found. The available English abstract does not explicitly list pseudoionone but it is said that the test compounds "(I) or (II) [not otherwise described] prevents aquatic harmful creature (e.g. oyster, sea mussel, barnacle, laver [possibly larvae], slime from adhering to ships bottoms, cooling water tubes, submarine constructions etc. (I) has a low toxicity to humans, animals and fish and edible shellfish. (I) or (II) shows high controlling effect at very low concentration. Since (I) or (II) can be readily decomposed by bacteria in seawater, it does not pollute the environment."
No further information is given.
Conclusion: Possibly pseudoionone is readily degradable in seawater.
Reliability: (4) not assignable
No concise information, link to pseudoionone through CAS number only, reliability 4.

24-JUN-2003 (60)

Type: aerobic
Inoculum: other: 50% activated sludge from municipal sewage works, 50% from in-house pilot industrial sewage works
Concentration: 30 mg/l related to Test substance
Contact time: 28 day(s)
Degradation: = 97 % after 28 day(s)
Result: inherently biodegradable
Deg. product: not measured

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

Remark: Corroborating data, read-across to structurally closely related substance.
Result: beta-Ionone was well inherently biodegradable in this test measuring oxygen consumption.
Test substance: beta-Ionone, CAS 14901-07-6, closed-ring isomer of pseudoionone.
Conclusion: The closely related substance, beta-ionone (CAS 14901-07-6), is well inherently biodegradable.
Reliability: (2) valid with restrictions
In this "ecotoxicological assessment", prepared for purely in-house use, only very bare data are given. However, the lab routinely produced such "ecotoxicological assessments" according to highly standardised, but non-GLP procedures, the reliability is accepted as 2.
19-JUN-2003 (37)

3.6 BOD5, COD or BOD5/COD Ratio

3.7 Bioaccumulation

Species: other: bioconcentration factor

Method: other: calculated
Year: 2003
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Result:

Calculated BCF	Estimation Program	Source
239.9	BCFWIN v2.14	EPISuite v.3.10
478	ACD Solaris V4.67	SciFInder
500	ChemSCORER beta100	ChemSCORER
501	EUSES	EUSES v.1.0

Conclusion: Based on two calculated bioconcentration factors, in the absence of information on metabolism or excretion, pseudoionone is predicted to bioconcentrate moderately.
Reliability: (2) valid with restrictions

QSAR values, commonly accepted, reliability 2.
10-JAN-2006 (16) (23) (25) (87)

Species: other: Overall bioaccumulation factor

Method: other: calculated
Year: 2003
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Result: An overall bioaccumulation factor of 1647 for lake trout, comprising both biomagnification through a sediment and a water foodnet and bioconcentration from water, was calculated for pseudoionone, based on physicochemical basic data.
Reliability: (4) not assignable

Unvalidated computer model, reliability 4.
10-JUN-2003 (16)

3.8 Additional Remarks

Memo: Predicted fate in a sewage works

Result: The behaviour and fate of pseudoionone in sewage works was modelled using various programs. Entering basic phyisco-chemical properties and the nearly ready biodegradability (except for the 10-day-window criterion), respectively the corresponding parameters according to the documentation or help for the respective programs, the following predictions in per cent of influent were derived:

Programs	STP v1.50 a)	STP/ EPISuite a)	SimpleTreat v3.1 b)	c)	USES v3.0 d)
Influent	100	100	100	100	100
Sludge adsorption	15.2	5.9	18.4	16.7	17.3
Biodegradation	80.1	92.2	41.0	64.1	55.2
Volatilisation	0.02	0.0	0.2	0.1	0.2
Total removal	95.2	98.1	59.6	81.0	72.7
Effluent	4.8	1.9	40.4	19.0	27.3

a) Settings: half-life in primary settler = 30 h, in aeration and final settler = 3 h.
b) Biodegradation constant k = 0.3/h (EU Technical Guidance Document default), with primary sedimentation.
c) Biodegradation constant k = 1.0/h (upper SimpleTreat limit), with primary sedimentation.
d) Selected "readily biodegradable, failed 10-day-window".

Conclusion: Wastewater treatment plant modelling suggests a high rate of degradation of pseudoionone in sewage works, from just below 59.6% to 98.1%. The fraction adsorbing to sludge is predicted to range between 5.9% and 18.4%, while the effluent range is the widest with 1.9% to 40.4%.
One experimental datum with pseudoionone distillation aqueous residues from Teranol Lalden, with an unspecified mixture of solvents, pseudoionone and by-products, showed 98% elimination in an inherent laboratory test (see chapter 1.10, Source of Exposure), giving some support for the higher-degradability predictions for pseudoionone.

Reliability: (4) not assignable
Computer models, reliability 4.
16-JUN-2003 (23) (88)

4. AQUATIC ORGANISMS

4.1 Acute/Prolonged Toxicity to Fish

Type: static
Species: Leuciscus idus (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no
NOEC: <= 4.64 - measured/nominal
LC0: = 4.64 - measured/nominal
LC50: = 6.8 - calculated
LC100: = 10 - measured/nominal
Limit Test: no

Method: other: DIN 38412, part 15: Determination of the effects of substances in water on fish
Year: 1989
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Animals and keeping
Golden orfe (Leuciscus idus), obtained from Fischzucht Paul Eggers (Hohenwestedt, Germany) on 21-Feb-1989. Fish were kept in activated-carbon-filtrated flow-through tap water at 20-21 °C, with light oil-fee-air aeration and a photoperiod of 16 hours light/8 hours dark for approximately 4 weeks before start of test. They were fed Growing Feed F/B 50 (SSNIFF Spezialdiäten GmbH, Soest, Germany). At the start of the test the fish were on average 6.0 (range 5.5-71.) cm long and weighed on average 1.8 (1.2-2-8) g.
Medium and test substance concentrations
For the toxicity study the medium was reconstituted freshwater according to DIN Guideline 38412, part 11 (full details in report).
All-glass aquaria of approximately 15 l volume were used for the test. Aquaria were filled with 10 l of reconstituted medium, weakly aerated and left to equilibrate temperature and dissolved oxygen during 3 days.
Based on range-finding pretests with an LC50 of approximately 10 mg/l nominal concentration, loading concentrations of 100, 46.4, 21.5, 10.0 and 4.64 mg/l (all nominal concentrations) plus 0 mg/l (controls) were selected. For test concentrations, the corresponding amount of pseudoionone was added to the experimental tanks containing 10 l of medium without any further pretreatment or emulsifier. Subsequently 10 fish were added per tank respectively concentration including controls. The fish were observed at 1, 24, 48, 72 and 96 hours after introduction, dead fish were removed and signs and symptoms recorded according to an internal list. Also, other remarks were noted if appropriate. The test lasted for 96 hours without exchange of medium.
Experimental data allowing, the median lethal concentration would be computed by probit analysis according to Finney.

Result: Mortalities during the adaptation period were in a normal range.
During the test the following mortalities were recorded:

Concentration mg/l nominal	Fish n	Dead fish, n, after 1 h	24 h	48 h	72 h	96 h
0 (controls)	10	0	0	0	0	0
4.64	10	0	0	0	0	0

10.0	10	0	7	10	10	10
21.5	10	0	10	10	10	10
46.4	10	0	10	10	10	10
100.0	10	0	10	10	10	10

At 24 hours, a "narcotic-like state" was noted for the remaining fish in 10 mg/l nominal concentration.
During the test, undissolved test substance was visible on the water surface.
The pH is given as approximately 8.0 in all aquaria, more than 60% ogygen saturation was recorded for all measurements and the temperature during the whole test was between 20 and 21 °C.

Test substance: Pseudoionone from BASF AG, purity >94%. Further "details on the characterization are included in the raw data" (test report, page 2).

Conclusion: Pseudoionone was toxic to fish at concentrations above 4.64 mg/l nominal (LC0), with the LC100 being reached at 10 mg/l nominal already within 24 hours. The LC50, as determined by geometric mean, is 6.8 mg/l nominal, however, it must be noted that this derivation is not in the original report but was made based on the original data.
The description of symptoms of the remaining fish in 10 mg/l nominal at 24 hours hints at narcosis as the toxic mechanism.

Reliability: (2) valid with restrictions
Short but detailed report from a professional industry ecotoxicology laboratory, with full methods and data. Reliability 2.

Flag: Critical study for SIDS endpoint

10-JAN-2006 (58)

Type: static
Species: Oncorhynchus mykiss (Fish, fresh water)
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no
LC0: = 5 - measured/nominal
LC50: = 7.1 - calculated
LC100: = 10 - measured/nominal
Limit Test: no

Method: other: internal 48-hour acute fish toxicity test
Year: 1989
GLP: no
Test substance: other TS

Remark: Corroborating data, read-across to structurally closely related substance.

Result: Fish showed non-lethal effects at 5 mg/l and all died at 10 mg/l. The geometric-average LC50 is 7.1 mg/l

Test substance: beta-Ionone, CAS 14901-07-6, closed-ring isomer of pseudoionone.

Conclusion: The closely related substance, beta-ionone (CAS 14901-07-6), has a geometrically interpolated 48-hour acute fish toxicity of 7.1 mg/l, which is very close to the LC50 of pseudoionone of 6.8 mg/l.

Reliability: (2) valid with restrictions
In this "ecotoxicological assessment", prepared for purely in-house use, only very bare data are given. However, the lab routinely produced such "ecotoxicological assessments" according to highly standardised, but non-GLP procedures, the reliability is accepted as 2.

19-JUN-2003 (37)

Type: other: QSAR calculation
Unit: mg/l **Analytical monitoring:**
LC50: = 3.12 - calculated

Method: other: QSAR calculation
Year: 2003
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: The QSAR formula for non-polar narcosis (baseline toxicity, minimum toxicity) for fish and 96 hours according to the EU Technical Guidance Document was used, using the molecular weight of 192.30 and the average experimental logPow of 4.0 and assuming nonpolarity for pseudoionone:
logLC50 (mol/l) = -0.85 logKow - 1.39
Result: QSAR LC50 (fish, 96 h) = 3.12 mg/l
Conclusion: As the calculated non-polar baseline fish toxicity of 3.12 mg/l is in the same dimension as the experimental interpolated LC50 of 6.8 mg/l, it is judged that pseudoionone acts by baseline or minimum toxicity and not through any receptor-mediated process.
Reliability: (2) valid with restrictions
QSAR published by EU technical Guidance Document, reliability 2.

15-JAN-2004 (18)

4.2 Acute Toxicity to Aquatic Invertebrates

Type: static
Species: Daphnia magna (Crustacea)
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no
NOEC: = .58 - measured/nominal
EC0: = 1 - calculated
EC50: = 3.7 - calculated
EC100: = 10 - measured/nominal
Limit Test: no

Method: other: DIN 38412 part 11, Acute toxicity of substances in water to daphnia
Year: 1989
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Animals and keeping
Daphnia magna, originally from the Bundesgesundheitsamt (Berlin, Germany), were bred at the test laboratory. They were fed green algae (Scenedesmus subspicatus) once daily, kept at 21 °C in daphnid medium according to DIN 38412 part 11, 8.2, with daily water exchanges except on weekends. Young daphnids were used for the test.
Test solutions
A 100-mg/l stock solution was prepared with water and Cremophor emuslifier. A range-finding pretest had shown toxicity below 10 mg/l nominal concentration. Based on this, definitive test concentrations were prepared with the stock solution diluted with daphnid medium, at the following nominal

concentrations: 0.58, 1.0, 1.8, 3.2, 5.8 and 10.0 mg/l as well as 0 mg/l (blank control) and 0 mg/l plus Cremophor as in the higest test concentration (emulsifier control).
Test
The test was performed from 06-Dec-1988 to 08-Dec-1988. All concentrations including both controls were tested in quadruplicate. 5 young daphnids each were added to 50 ml of the respective solutions and kept at a constant 21 °C, with pH and dissolved oxygen monitored, for 48 hours. Daphnids were inspected at 3, 6 24 and 48 hours after the start of the test. Immobilised animals were checked according to the DIN guideline and recorded.
Statistical evaluation
The Spearman-Karber correlation was used for calculation of the EC50 and the 95% confidence interval, based on the pooled data from the four quadruplicates of each concentration. Additionally, the toxicity was graphically shown on log-probit paper.

Result:

Concentration mg/l nominal	Immobilised daphnids (% of all quadruplicates) 3 h	6 h	24 h	48 h
10.0	5	20	75	100
5.8	0	0	45	70
3.2	0	0	5	50
1.8	0	0	0	0
1.0	0	5	5	5
0.58	0	0	0	0
0 (blank control)	0	0	0	0
0 (Cremophor control)	0	0	5	5

Statistical 48-hour EC50 = 3.7 mg/l nominal, 95% confidence interval = 3.1-4.4 mg/l.
Test considered valid because 1) < 10% mortality in controls, 2) dissolved oxygen concentration >= 8 mg/l (minimal requirement 2 mg/l), 3) pH remained between 7.5 and 7.8 in all vessels, 4) temperature was constant at 21 °C and 5) a reference test with the same breeding strain of daphnids, performed 2 days before the pseudoionone test, showed an EC50 of 1.09 mg/l for potassium dichromate.

Test substance: Pseudoionone from BASF, no data regarding purity.

Conclusion: In an acute 48-hour static daphnid toxicity test based on loading respectively nominal concentrations with an emulsifier, the NOEC was 0.58 mg/l, the statistical EC0 is given in the test report as 1.0 mg/l, the statistical EC50 is given in the test report as 3.7 mg/l and the EC100 was 10 mg/l. At 10.0, 5.8 and 3.2 mg/l nominal concentration the magnitude of effects on daphnids increased over time. Pseudoionone proved toxic to daphnids.

Reliability: (2) valid with restrictions
Brief but detailed test report from a contract laboratory. All single data and test circumstances given. Reliability 2.

Flag: Critical study for SIDS endpoint

22-JAN-2003 (73)

Type: other: QSAR calculated
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:**
EC50: = 1.46 - calculated

Method: other: QSAR calculated
Year: 2003
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: The QSAR formula for non-polar narcosis (baseline toxicity, minimum toxicity) for daphnia and 48 hours according to the EU Technical Guidance Document was used, using the molecular weight of 192.30 and the average experimental logPow of 4.0 and assuming nonpolarity for pseudoionone:
logEC50 (mol/l) = -0.95 logKow - 1.32.
Result: QSAR EC50 (daphnia, 48 h) = 1.46 mg/l
Conclusion: As the calculated non-polar baseline daphnid toxicity of 1.46 mg/l is in the same dimension as the experimental EC50 of 3.7 mg/l, it is judged that pseudoionone acts by baseline or minimum toxicity and not through any receptor-mediated process.
Reliability: (2) valid with restrictions
QSAR published by EU technical Guidance Document, reliability 2.
15-JAN-2004 (18)

Type: other: inhibition of larval adhesion to substrate, no other information
Species: other: "oyster, sea mussel, barnacle, laver [?larvae?], slime"
Unit: **Analytical monitoring:** no data

Method: other: no data
Year: 1982
GLP: no data
Test substance: no data

Remark: Kuraray declined to give further information on this patent.
Result: By searching for the CAS number 141-10-6, a location of a Japanese patent was found. The available English abstract does not explicitly list pseudoionone but it is said that the test compounds "(I) or (II) [not otherwise described] prevents aquatic harmful creature (e.g. oyster, sea mussel, barnacle, laver [possibly larvae], slime from adhering to ships bottoms, cooling water tubes, submarine constructions etc. (I) has a low toxicity to humans, animals and fish and edible shellfish. (I) or (II) shows high controlling effect at very low concentration. Since (I) or (II) can be readily decomposed by bacteria in seawater, it does not pollute the environment." No other information is given.
Conclusion: Possibly, pseudoionone is toxic to various larvae of marine invertebrates. Further, possibly pseudoionone is biodegradable in seawater.
Reliability: (4) not assignable

No concise information, link to pseudoionone through CAS number only, reliability 4.
11-JUN-2003 (60)

Species: Artemia salina (Crustacea)
Unit: **Analytical monitoring:** no data

Method: other: no data
Year: 1982
GLP: no data

Test substance: no data

Remark: This is a patent abstract that was located by searching with "pseudoionone" respectively "141-10-6". As the abstract only describes a different substance, it is not known whether and to which extent pseudoionone is also toxic to Artemia. Kuraray did not translate this patent application into English.

Result: Abstract text:
"Terpene ketones are antifouling agents. Thus, 50 ppm 6-methyl-8-(3',4'-dichlorophenyl)-3,6-octadien-2-one (CAS 82404-85-7) controlled Artemia salina by 100% in <=6 hours after application."

Conclusion: Based on a very short Japanese patent abstract in English, located by searching for the CAS number of pseudoionone, possibly pseudoionone is toxic to Artemia salina brine shrimps.

Reliability: (4) not assignable

01-DEC-2003 (61)

4.3 Toxicity to Aquatic Plants e.g. Algae

Species: Scenedesmus subspicatus (Algae)
Endpoint: other: biomass and growth rate
Exposure period: 72 hour(s)
Unit: mg/l **Analytical monitoring:** no
LOEC: = .5 - measured/nominal
EC10: = .525 -
EC50: - calculated
EbC50 : = 1.11 - calculated
ErC50 : = 2.02 - calculated
EC100 : = 10 - measured/nominal
Limit Test: no

Method: other: DIN 38412, part 9
Year: 1989
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: An algal growth inhibition test was performed at Dr. U. Noack Laboratorium according to DIN Guideline 38412, part 9.
Algal species
Scenedesmus subspicatus CHODAT were originally obtained from Algensammlung Göttingen, Germany, and were maintained in artificial algal medium according to DIN 38412.
Test procedure
A pretest with 0.5, 5, 50 and 500 mg pseudoionone/l medium with Cremophor as an emulsifier at 10% of the pseudoionone concentration had resulted in the following selection of main test concentrations (also with Cremophor at 10% of substance concentration): 0.5, 1.0, 2.5, 5.0, 10.0, 0.0 (blank control) and 0.0 (maximal Cremophor control) mg/l nominal concentration. Potassium dichromate was used as a reference substance.
Test concentrations including both controls and the reference were set up in quadruplicate each according to the DIN guideline. The test was run over a total of 96 hours under illumination, from 13-Nov-1989 to 17-Nov-1989. The pH was determined in every single vessel at the start and at the end of the test, temperature was kept in the range of 21-25 °C.

Density of algae was determined using fluorimetry at the start and then after every period of 24 hours; additionally, possible autofluorescence was also checked.
After the test the four values per concentration and time point were averaged and statistics performed according to Tallarida & Jacob (The dose-response relation in pharmacology. Springer 1979, pp. 98-103).

Result:

Concentration mg/l nominal	Cell density, n*1000/ml, at time 0 h	24 h	48 h	72 h	96 h
0.5	12	40	146	362	887
1.0	12	28	92	245	628
2.5	12	13	32	107	194
5.0	12	12	24	55	109
10.0	12	5	7	6	5
0 (blank control)	12	41	147	409	918
0 (Cremophor control)	13	49	180	407	912

Fluorimetry showed that the Cremophor emulsifier in the highest concentration had some autofluorescence, which was deducted from results, but that pseudoionone itself had no autofluorescence. There was no effect on pH that might have biased the results nor was there any influence on photosynthetic capacity of the algae.
Direct evaluation

Concentration mg/l nominal	Inhibition, %, at 72 h biomass	growth rate
0.5	16.7	1.1
1.0	47.5	12.4
2.5	82.7	36.5
5.0	90.1	55.8
10.0	100.0	100.0
0 (blank control)	0	0
0 (Cremophor control)	0	0

Statistical evaluation (95% confidence interval in brackets)

	72 h	96 h
EbC50, mg/l nominal	1.107 (0.37-3.29)	1.261 (0.48-3-32)
EbC10, mg/l nominal	0.525 (0.11-2.43)	0.625 (0.17-2.36)
ErC50, mg/l nominal	2.018 (0.71-5.76)	2.623 (1.21-5.67)
ErC10, mg/l nominal	1.085 (0.31-3.85)	1.655 (0.75-3.68)

The pH at the start of the test ranged between 7.76 and 7.84 in the different flasks, at the end of the test it ranged between 7.75 and 8.83, the temperature during the test is given in-between 20 and 21 °C.

Test substance: Pseudoionone from BASF AG, production date 14-Feb-1989; no indication of purity. Product specification sheet filled in by sponsor (BASF), attached to algal test report.

Conclusion: In a an algal growth inhibition test, pseudoionone with Cremophor as an emulsifier had a 72-hour EbC50 of 1.1 mg/l and a 72-hour ErC50 of 2.0 mg/l, both nominal concentrations. Biomass was slightly but significantly inhibited at the lowest concentration tested (0.5 mg/l nominal) while growth rate was not. Over 96 hours, both EbC50 and ErC50 were slightly higher.

Reliability: (2) valid with restrictions
Brief but detailed test report from a professional contract laboratory, with full data. Reliability 2.

Flag: Critical study for SIDS endpoint

23-MAR-2004 (72)

Species: other algae: QSAR calculation
Endpoint: other: growth
Unit: mg/l **Analytical monitoring:**
EC10: - calculated
EC50: = 1.13 -

Method: other: QSAR calculation
Year: 2003
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: The QSAR formula for non-polar narcosis (baseline toxicity, minimum toxicity) for algae and 72-96 hours according to the EU Technical Guidance Document was used, using the molecular weight of 192.30 and the average experimental logPow of 4.0 and assuming nonpolarity for pseudoionone:
logEC50 (mol/l) = -1.00 logKow - 1.23.
Result: QSAR EC50 (algae, 72-96 h) = 1.13 mg/l
Conclusion: As the calculated non-polar baseline algael toxicity of 1.13 mg/l is in the same dimension as the experimental EC50 of 1.1 mg/l, it is judged that pseudoionone acts by baseline or minimum toxicity and not through any receptor-mediated process.
Reliability: (2) valid with restrictions
QSAR published by EU technical Guidance Document, reliability 2.
15-JAN-2004 (18)

Species: other algae: Synechococcus sp. 6911 (Institut Pasteur, Paris), blue-green algae (Cyanobacteria)
Endpoint: growth rate
Exposure period: 24 hour(s)
Unit: mg/l **Analytical monitoring:** no data
LOEC: = 3 - measured/nominal
Limit Test: no

Year: 1982
GLP: no data
Test substance: no data

Method: Blue-green algae of the strain Synechococcus 6911 from the Institut Pasteur, Paris, France, were incubated in a synthetic nutrient broth with additional NaHCO3 according to [Z Naturforsch (1976): 31c: 491]. Test substances including pseudoionone, dissolved in ethanol, were added at to final concentrations of 100, 75, 50, 20, 15, 7.5, 5 and 3 ppm. Algae were added at a starting density of 5*10E7 cells/ml of broth at a test volume of 100 ml per experiment in 300-ml glass vessels, which were incubated on a shaker at 27 °C and with an illumination intensity of 1000 lux. After 24 hours of incubation the cell density per concentration respectively control was measured to determine the minimal inhibitory concentration or LOEC.
Remark: Please see also chapter 4.4, Toxicity to Micro-Organisms, e.g., Bacteria, where the same test is described with in detail with a focus on biochemical endpoints (Jüttner & Bogenschütz, 1983).
Result: Pseudoionone is listed to inhibit the growth of Synechococcus 6911 at a minimal tested concentration of 3ppm or approximately 3 mg/l.

Test substance: Various terpenoid test substances including pseudoionone, from BASF, Germany. No further data on purity of test substances.
Reliability: (4) not assignable
No detailed information on test substance, no information on controls/solvent controls, only MIC given without quantification of effects. Reliability hard to assess, tentatively 4, possibly better.

28-NOV-2003 (51)

4.4 Toxicity to Microorganisms e.g. Bacteria

Type: aquatic
Species: activated sludge, domestic
Exposure period: 30 minute(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: > 1000 - calculated
EC20 : ca. 300 - calculated

Method: OECD Guide-line 209 "Activated Sludge, Respiration Inhibition Test"
Year: 1988
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: A bacterial respiration inhibition test was performed according to OECD Guideline 209 and ISO Guideline 8192 with pseudoionone. The baseline oxygen consumption of 1 g (dry weight)/l activated sludge from a municipal wastewater treatment plant was compared to that of the same sludge concentration with added pseudoionone over short-time exposure of 30 min. According to the guideline, a statistical inhibition of 20% (EC20) was defined as the toxic threshold concentration.
Result: Blank respiration rate after 30 minutes: 26 mg/(l*h)
EC20 (30 min) ca. 300 mg/l (nominal concentration)
EC50 (30 min) > 1000 mg/l (nominal concentration)
EC80 (30 min) > 1000 mg/l (nominal concentration)
Test substance: Pseudoionone from BASF AG, batch and purity not stated.
Conclusion: Pseudoionone has a high toxic threshold concentration of approximately 300 mg/l (nominal concentration). Therefore, no risk to wastewater treatment plants is foreseen from pseudoionone.
Reliability: (2) valid with restrictions
Reprint of test report based on original laboratory data, with full results, from a professional industry biodegradation laboratory, following international guidelines. Reliability 2.
Flag: Critical study for SIDS endpoint

07-JAN-2003 (76)

Type: other: inhibition of growth and carotenogenesis in photosynthetic cyanobacteria
Species: other bacteria: Synechococcus, strain PCC6911, Cyanobacteria
Exposure period: 42 hour(s)
Unit: mg/l **Analytical monitoring:** no
NOEC: = 2 - measured/nominal
LOEC : = 3 - measured/nominal

Year: 1983
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: The biological effects of 20 geranyl derivatives, including pseudoionone, on carotenoid biosynthesis of the marine cyanobacterium Synechococcus PCC6911 was tested.
Test system
A starting culture of Synechococcus PCC6911 was obtained from the Pasteur Culture Collection (PCC; Paris, France). The cyanobacteria were cultivated in 300-ml Erlenmeyer flasks at 27 °C under fluorescent lighting (1400 lx)on a shaking table (120 strokes/min) in medium as described [Jüttner et al. (1983); Gen Microbiol 129: 407 ff], which was indirectly supplied with a 0.27% v/v Carbon-dioxide/air mixture at 450 ml/min.
Cyanobacterial assay
Synechococcus cultures at the end of the exponential growth stage were diluted with fresh medium supplemented with 20 mM NaHCO3 to give a starting test concentration as measured by chlorophyll a of 0.3 µg/ml. 100-ml samples of diluted suspension were transferred to Erlenmeyer flasks with ground glass stoppers under axenic conditions and incubated as above. Various amounts of 10% stock solutions in ethanol of test substances, including pseudoionone, were added to the flasks. The highest equivalent of pure ethanol was included in the untreated control cultures. Growth rates were determined by optical density at 550 nm in a Zeiss PM2K spectrophotometer twice daily.
Separation and quantitative determination of pigments
Chlorophyll a and the total carotenoids were determined in an ethanolic extract obtained from 5 ml of cyanobacterial suspension. Chlorophyll a was determined quantitatively by the molar extinction coefficient of Seely and Jensen [Spectrochim Acta 21: 1835 ff, 1965] and total carotenoids by the equation "carotenoids (nmol/ml) = 8.27*A(477) - 0.19*A(665)". For the determination of individual carotenoids, 195-ml samples were necessary, which were extracted and separated on Kieselgel G (Merck) plates as described [Jüttner F (1979): Z Naturforsch 34C: 957 ff]. Phytofluene (15-cis-7,7',8,8',11,12-Hexahydro-psi,psi-carotene, CAS 27664-65-9, a direct precursor of zeta-carotene and lycopene) was determined by fluorimetry in a Perkin Elmer MPF-3 with excitation wavelength 366 nm and emission wavelength 490 nm, in a carotene fraction eluted from an Al2O3 column with light petroleum containing 2% diethylether.

Result: Growth
Growth of the cultures was followed for up to 180 hours, up to 4 generations. When inhibitory compunds were applied, eg, pseudoionone, low concentrations did not affect the growth rate during the first two generations, however, high degress of inhibition were noted at later stages. By increasing the concentrations, the inhibition stage was shifted to earlier times. For pseudoionone, the NOEC for growth rate was 2 ppm, while the LOEC was 3 ppm. At a concentration of 3 ppm pseudoionone, the growth as determined by optical density was indistinguishable from controls up to 24 hours, but then started to decline.
Chlorophyll a and carotenoids respectively precursors
Exposure to 3 ppm pseudoionone did not have any influence on

chlorophyll a biosynthesis up to 42 hours, the limit of this part of the test. However, formation of total carotenoids was slightly reduced compared to controls already at 8 hours, with reduction becoming stronger over time. Again with 3 ppm pseudoionone, biosynthesis of phytofluene reached a plateau at 16 hours and the measured concentration of phytofluene remained at this plateau of approximately 4 nM. zeta-Carotene, the further carotenoid intermediate from phytofluene, continued rising up to approximately 13 nM at 24 hours, where it more or less remained with approximately 11 nM at 42 hours. Both phytofluene and zeta-carotene were undetectable in exponentially growing control cultures. The onset of phytofluene accumulation could already be observed at 30 minutes of exposure.
The reversibility of the inhibition of further carotenoid synthesis by pseudoionone was demonstrated when pseudoionone was washed out with new medium after a 30-hour incubation, when growth rate and carotenoid synthesis re-approached that of control cultures and accumulated phytofluene and zeta-carotene were for the biggest part re-metabolised within 5-10 hours.

Test substance: Pseudoionone, mixture of cis/trans isomers, obtained from BASF AG, Ludwigshafen, Germany.

Conclusion: Pseudoionone inhibited the grwoth of Synechococcus PCC6911 cultures at concentration of 3 mg/l and higher. Pseudoionone had no influence on chlorophyll a biosynthesis, but it did inhibit carotene formation. The rapid accumulation of the carotenoid precursors phytofluorene and zeta-carotene was interpreted by the authors to argue for direct interaction of pseudoionone with the enzymes that convert both phytofluorene and zeta-carotene.

Reliability: (2) valid with restrictions
Detailed methods and analyses, results clearly presented as informative graphs. Reliability 2.

28-NOV-2003 (52)

Type: soil
Species: other fungi: Candida albicans, Phoma betae, Geotrichum candidum, Oospora lactis
Exposure period: 5 day(s)
Unit: **Analytical monitoring:** no

Year: 1960
GLP: no
Test substance: no data

Method: The vapours of 196 chemicals were tested in vitro against growing cultures of Candida albicans ATCC 10231, Phoma betae ATCC 6504, Geotrichum candidum Coll. No. 4762 and Oospora lactis ATCC 4798. All test organisms were cultivated on Sabouraud maltose broth at 22 °C and transferred every five days.
For the test dishes, 15 ml of Sabouraud maltose agar were poured into Petri dishes and allowed to harden. The test cultures were shaken by hand several times to distribute evenly the mycelia and spores. the surface of the hardened agar was streaked with 0.5 ml of a 5-day-old broth culture of the respective test organism.
Aluminium cups (20 mm diameter, 5 mm deep) containing 0.5 mlof the respective chemical were placed in the centre of the Petri

dish top, Then the agar-plated and seeded bases were inverted on the tops, the chemical was about 5 mm from the agar surface above it. Vapours of the chemicals were then allowed to emanate throughout the 5-day incubation period at 22 °C. All chemicals were tested in triplicate with one cup per Petri dish.
After incubation, the presence of a definite clear zone of inhibition on the agar surface indicated that the vapour possessed antifungal activity and the larger the zone the greater the activity in this test system. Measured inhibition zones were averaged for the respective test chemical.

Result:

	Average diameter of inhibition zone, mm			
	C.albicans	P.betae	G.candidum	O.lactis
Pseudoionone	0	20	0	0

	Relative sensitivity of fungi, % inhibition of all chemicals			
	53%	60%	53%	53%

Pseudoionone vapours caused inhibition only on P. betae, with an average growth-free ring of 20 mm diameter, but not on the other fungi. Comparing the fungi for sensitivity, it was shown the P. betae was the most sensitive while all others showed lower but comparable sensitivity.

Conclusion: Pseudoionone vapours caused slight growth inhibition in one out of four tested fungal species, Phoma betae, which proved to be the species with the highest sensitivity among those tested. Pseudoionone did not cause any inhibition in the other three species.

Reliability: (2) valid with restrictions

Old (1961) paper, no details as to substances and results given only as a table, but clear methods and clear presentation of data. Reliability tentatively assigned 2.

04-JUN-2003 (67)

Type: other: growth and carotenoid biosynthesis in a mould
Species: other fungi: Phycomyces blakesleeanus
Exposure period: 60 hour(s)
Unit: mg/l **Analytical monitoring:** no
EC50: < 220 - measured/nominal
LOEC : ca. 22 - measured/nominal

Year: 1952
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: Test system
Phycomyces blakesleeanus was grown in a medium containing 3 g Difco yeast extract, 25 g glucose and 6 mg thiamine per litre, on filter paper supported by glass beads in Petri dishes with 20 ml medium. Cultures were inoculated and grown under illumination at approximately 25 °C for 36 hours, when aerial mycelium is thick but short, with no fruiting bodies and negligible pigmentation.
Test procedure
Approximately 5 µl of test substance, including pseudoionone, was added to these pre-grown cultures, which were subsequently kept in the dark for a further 24 hours. The end points were further growth, development of fruiting bodies and of

pigmentation as determined by colour. The experiment was repeated with one-tenth the original test substance concentrations.

Result: With approximately 5 µl pseudoionone/20 ml medium (ca. 250 µl/l, corresponding to ca. 220 mg/l using a density of 0.895), Phycomyces cultures "showed little development and no pigment production". With one-tenth that concentration, ca. 22 mg pseudoionone/l, growth is reported to be "nearly normal" but total pigment (beta-carotene plus lycopene) production was still slightly reduced to 88% of controls. However, "spectroscopic and chromatographic evidence indicated a small increase in the absolute amount of lycopene for [citral] and and [pseudoionone], compared with [beta-ionone] and the control."

Test substance: Pseudoionone, synthesised by the authors by coupling citral with acetone.

Conclusion: Both growth and beta-carotene and lycopene biosynthesis of the mould Phycomyces blakesleeanus are inhibited in the presence of ca. 220 mg/l pseudoionone in the medium, while the addition of beta-ionone, which is closely related to pseudoionone but has a closed ring, to the culture medium enhances beta-carotene production, which is a carotene with closed terminal rings.
At ca. 22 mg pseudoionone/l medium, growth was nearly normal and pigment production was nearly as high as in controls. Compared to controls, however, chromatography showed a small increase in lycopene, which is a carotene with open terminal rings, similar to pseudoionone. The authors conclude that biosynthesis of lycopene and beta-carotene in Phycomyces is markedly influenced by use and concentration of compounds "presumably providing terminal groups in the carotenoid molecule".

Reliability: (4) not assignable
Clear description of culture but no details on chromatography and quantification and only very summary results, hence reliability cannot be properly assessed.

01-DEC-2003 (69)

Type: other: toxicity against bacteria and fungi

Result: A patent for a "veterinary disinfectant containing ionone and terpene" describes a mixture that "comprises about 45% ionone, about 40% another terpene, about 20% surfactant, and about 5% iso-Pr alc". This preparation is "effective against several types of bacteria and a broad range of fungi, and is esp. useful in veterinary medicine for control of foot diseases. [...] As a foot bath, the compn. is dild. with water about 1 to 1,000, and as a spray, it is dild. with water or org. solvent about 1:1 to 1:100. Preferred ionones are beta-ionone and pseudoionone."

Conclusion: Pseudoionone is described as an antibacterial and antifungal compound in a mixture with other terpenes, surfactant and isopropanol.

Reliability: (4) not assignable
Secondary source: SciFinder bibliographic information, online. Reliability 4.

15-JAN-2004 (36)

Type: aquatic

Species: activated sludge
Exposure period: 28 day(s)
Unit: mg/l **Analytical monitoring:** no
NOEC: = 30 - measured/nominal

Method: other: inherent respirometric test
Year: 1989
GLP: no
Test substance: other TS

Result: No inhibition of co-substrate degradation respectively toxicity to activated sludge micro-organisms was seen in this inherent repsirometric test.
Test substance: beta-Ionone, CAS 14901-07-6, closed-ring isomer of pseudoionone.
Conclusion: The pseudoionone isomer, beta-ionone, was not toxic to aeriobic activated sludge bacteria at a concentration of 30 mg/l.
Reliability: (2) valid with restrictions
In this "ecotoxicological assessment", prepared for purely in-house use, only very bare data are given. However, the lab routinely produced such "ecotoxicological assessments" according to highly standardised, but non-GLP procedures, the reliability is accepted as 2.
24-JUN-2003 (37)

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

Species: other: Caenorhabditis elegans (Nematoda), common soil and sediment invertebrate
Endpoint: other: growth, egg production, fertility
Expos. period: 72 other: hours
Unit: mg/kg sediment dw
NOEC: = 100 - measured/nominal
EC50 : = 821 - calculated

Method: other: test conforms to recent DIN draft with the exception of duration (3 days vs 4); the DIN draft was published after this test was performed.
Year: 2002
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: Test institution
Ecossa, Ecological Sediment and Soil Assessment, is a company founded by Dr Sebastian Höss in Munich, Germany. Dr Höss did his PhD on sediment testing using nematodes, he co-developed the published protocol for this test (see reference

Traunspurger et al., 1997) and he has years of experience with this type of testing.
Test animals
Caenorhabditis elegans is a common soil and sediment nematode that feeds on bacteria. Caenorhabditis are mostly (>99.9%) self-fertilising hermaphrodites, only <0.1% are males capable of fertilising hermaphrodites. The animals pass through 4 juvenile stages with moults to reach adult stage, self-fertilise and develop eggs in their body. At room temperature a full reproductive cycle takes about 72 hours. They can be easily grown and maintained as stock cultures on Petri dishes on agar plates with a bacterial lawn for food. They can be selected and synchronised to obtain juveniles of the first stage (J1), which were used in the tests. Test animals were fed on cultures of the bacterium Escherichia coli (OP50 strain).
Artificial sediment
An artificial sediment containing 30% dry sediment mix and 70% M9-medium (mostly water) was used for the test. Briefly, quartz sand, calcitic sand, kaolin, dolomite sand, ground sphagnum peat, iron(III) oxide and aluminium(III) oxide (all sources listed in report) were mixed in adequate proportions to result in an artificial sediment mix made up of 44% sand fraction, 48% silt fraction and 8% clay fraction and containing 2% organic substances.
Media
M9-medium was made up of 6 g Na2HPO4/l, 3 g KH2PO4/l, 5 g NaCl/l, 0.25 g MgSO4*7H2O/l and 1 ml/l of a cholesterol stock solution, consisting of 5 g cholesterol in 1 l of absolute ethanol. M9-medium was made up to 1 l using distilled water.
Food medium for E. coli bacterial culture consisted of 10 g peptone from casein/l, 5 g yeast extract/l and 10 g NaCl/l, made up with water.
NGM agar for E. coli bacterial culture consisted of 2.5 g peptone from casein/l, 17 g agar/l and 13 g NaCl/l; after mixing, autoclaving and cooling to approx. 55 °C, the follwing aliquots of sterile solutions are added: 1 ml cholesterol stock solution (see above), 1 ml 1M CaCl2 solution, 1 ml 1M MgSO4 solution and 25 ml 1MKH2PO4 solution, the latter adjusted to pH 6 using KOH.
Test setup
Test substances were dissolved in 96% ethanol in concentration series and 0.01 ml of the respective stock solution was thoroughly mixed with 0.75 g wet artificial sediment in the test vessels (Nunc polystyrene multiwells). Spiked sediments were left for 24 hours to allow equilibration of test substance between aqueous and solid phases. Before the start of the assay, 0.25 ml of bacterial suspension in double-concentrated M9-medium was added to each test well as food for the nematodes. After that, 10 juvenile worms of stage J1 were added by pipette to each well. Every test concentration including a vehicle control was run in triplicate for the range-finding test and in quintuplicate for the main test. The multiwell plates were incubated for 72 hours on a shaker at ±20 °C. Then, to stop the test, nematodes were heat-killed by warming the plates to approx. 55 °C, which makes them stretch, and stained with Rose Bengal dye. Nematodes were extracted from the sediment by centrifugation in a density gradient and parameters for the

endpoints were determined under a microscope at x100 and x400 magnification.
Endpoints
Parameters for the endpoints were as follows. Growth: length in µm; egg production: number of eggs in body; fertility: percentage of gravid worms (worms with >= 1 egg).
Statistics
One-way ANOVAS were carried out with the mean values of the replicates of the main test. In order to obtain NOEC and LOEC values, post-hoc tests according to Dunnett were performed additionally. For the determination of ECx values, dose-response curves (% inhibition vs control) were fitted to the respective data using a sigmoidal model.

Result: The range-finding pretest with concentrations from 50 to 5000 mg/kg sediment (dry weight) had shown no effect up to 100 mg/kg sediment. The main test was performed using concentrations of 0 (control), 100, 200, 400, 800, 1600, 3200 and 6400 mg pseudoionone/kg sediment (dry weight). At 200 mg/kg sediment there was a significant reduction in growth (-15.3%) and egg production (-38.8%) while fertility as measured by number of gravid worms was only significantly reduced (-55.9%) at 800 mg/kg sediment.
Observed and interpolated effect concentrations in mg/kg sediment (dry weight) are as follows:

Test parameter	NOEC	LOEC	EC50	EC90
Growth	100	200	2490	5183
Egg production	100	200	821	2893
Fertility	400	800	1537	3193

Test substance: Pseudoionone from Teranol, Lalden, Lot no. UU02033826, purity 96.1% (area, GC).

Conclusion: Pseudoionone is to be considered of relatively low toxicity to sediment-dwelling nematodes. The NOEC for pseudoionone was 100 mg/kg artificial sediment (dry weight) for two of three parameters, it was 400 mg/kg for the third (fertility). While effects on growth, egg production or fertility were observed at higher concentrations, the concentration-effect curves for all three parameters show a relatively flat slope. In view of the short reproduction time of Caenorhabditis, a very common sediment- and soil-dwelling nematode, this test also qualifies as a chronic study.

Reliability: (2) valid with restrictions
While the protocol is not an accepted OECD guideline and the institution is not GLP-approved, Dr Höss co-developed and refined the protocol, has a lot of experience with this type of testing which he does as a contract lab and presented a detailed report with all single basic data for the different concentrations tested (5 dishes with 10 animals each per concentration in the main test) plus full statistics for the whole test. Based on a clear protocol, careful documentation, testing in quintuplicate and full statistics, the report is judged to be of reliability 2.

Flag: Critical study for SIDS endpoint

01-DEC-2003 (46) (99)

4.6.2 Toxicity to Terrestrial Plants

Species: other terrestrial plant: "rice and other crops"

Year: 1981

GLP: no data
Test substance: no data

Result: By searching for the CAS number 141-10-6, a location of a Japanese patent was found. The available English abstract does not explicitly list pseudoionone but it is said that the "agricultural fungicide contains terpene type carbonyl cpd. of formula (I), (II) or (III) [no further information available] ... The active cpd. can be used for the protection of paddy rice, upland crops, fruit trees and wood from the attack of pathogenic fungi. ... The active cpd. shows excellent effect in the control of rice blast and rice helminthosporium leaf spot, and has no phytotoxicity to rice and other crops."
Conclusion: Possibly pseudoionone in combination with other terpene compounds works as a fungicide while being nontoxic to rice and other crops.
Reliability: (4) not assignable
No concise information, link to pseudoionone through CAS number only, reliability 4.
11-JUN-2003 (59)

4.6.3 Toxicity to Soil Dwelling Organisms

Type: other: artificial sediment
Species: other: Caenorhabditis elegans (Nematoda), common soil and sediment invertebrate
Endpoint: other: growth, egg production, fertility
Exposure period: 72 hour(s)
Unit: other: mg/kg artificial sediment (dry weight)
NOEC: = 100 - measured/nominal
EC50 : = 821 - calculated

Method: other: test conforms to recent DIN draft with the exception of duration (3 days vs 4); the DIN draft was published after this test was performed.
Year: 2002
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: Please see 4.6.1, Toxicity to Sediment-Dwelling Organisms.
Result: The range-finding pretest with concentrations from 50 to 5000 mg/kg sediment (dry weight) had shown no effect up to 100 mg/kg sediment. The main test was performed using concentrations of 0 (control), 100, 200, 400, 800, 1600, 3200 and 6400 mg isophytol/kg sediment (dry weight). At 200 mg/kg sediment there was a significant reduction in growth (-15.3%) and egg production (-38.8%) while fertility as measured by number of gravid worms was only significantly reduced (-55.9%) at 800 mg/kg sediment.
Test substance: Pseudoionone from Teranol, Lalden, Lot no. UU02033826, purity 96.1% (area, GC).
Conclusion: Pseudoionone is to be considered of relatively low toxicity to sediment- and soil-dwelling nematodes. The NOEC for pseudoionone was 100 mg/kg artificial sediment (dry weight) for two of three parameters, it was 400 mg/kg for the third (fertility). While effects on growth, egg production or fertility were observed at higher concentrations, the concentration-effect curves for all three parameters show a relatively flat slope. In view of the short reproduction time

of Caenorhabditis, a very common sediment- and soil-dwelling nematode, this test also qualifies as a chronic study.

Reliability: (2) valid with restrictions
Based on a clear protocol, careful documentation, testing in quintuplicate and full statistics, the test is judged to be of reliability 2.

Flag: Critical study for SIDS endpoint

11-JUN-2003 (46)

Type: other: no data
Species: other soil dwelling microorganisms: phytopathogenic fungi

Method: other: no data
Year: 1981
GLP: no data
Test substance: no data

Result: By searching for the CAS number 141-10-6, a location of a Japanese patent was found. The available English abstract does not explicitly list pseudoionone but it is said that the "agricultural fungicide contains terpene type carbonyl cpd. of formula (I), (II) or (III) [no further information available] ... The active cpd. can be used for the protection of paddy rice, upland crops, fruit trees and wood from the attack of pathogenic fungi. ... The active cpd. shows excellent effect in the control of rice blast and rice helminthosporium leaf spot, and has no phytotoxicity to rice and other crops."
No other information is given.

Conclusion: Possibly pseudoionone in combination with other terpene compounds works as a fungicide while being nontoxic to rice and other crops.

Reliability: (4) not assignable
No concise information, link to pseudoionone through CAS number only, reliability 4.

11-JUN-2003 (59)

4.6.4 Toxicity to other Non-Mamm. Terrestrial Species

Species: other not soil dwelling arthropod: Apis mellifica, honeybee
Endpoint: mortality
Expos. period: 24 hour(s)

Year: 1975
GLP: no data
Test substance: other TS

Method: Various substances were tested in the laboratory as potential honeybee-repellent additives to pesticides in order to reduce pesticide hazards to honeybees.
Tests were performed in 13x13x13 cm mesh cages with 25 worker bees per cage for 24 hours per test at 27±1 °C, 35-45% RH and a 12-h-light/12-h-dark cycle. Substances were tested at 5 concentrations at 3 replicates each, on bees from 3 colonies each. Control cages were tested in triplicate, too. Two test procedures were followed, gustatory repellence testing and spatial (olfactory) repellence testing. In total, 143 chemicals were tested for repellency to honeybees.
In the honeybee gustatory repellence test, where gustatory repellence and oral toxicity were assayed, the test substances

were prepared as 1% stock solutions. Serial dilutions were incorporated to 1:1 honey-water feeding mixtures, filled into vials and the vials capped with lids having 1.5-mm holes to allow the bees to feed. A similar vial, but without test substance in the feeding solution, was offered as an alternative, control feeding station.

Result: (E)-Pseudoionone is listed as "0", meaning nontoxic, in the results table. Hence, is is to be taken that no bees died during the 24 hours of exposure. However, no final concentration of (E)-pseudoionone in the feeding syrup is given.

Test substance: (E)-Pseudoionone, no further details given.

Reliability: (2) valid with restrictions
Detailed publication with clear (non-OECD) methods but only summary results, reliability 2.

Flag: Critical study for SIDS endpoint

04-JUN-2003 (3)

Species: other: Dysdercus cingulatus (Heteroptera, Pyrrhocoridae; red cotton bug), Tenebrio molitor (Coleoptera, Tenebrionidae; yellow mealworm), Musca domestica (Diptera, Muscidae; housefly), Aedes aegypti (Diptera, Culicidae; yellow fever mos

Endpoint: other: developmental inhibition

Year: 1978

GLP: no data

Test substance: no data

Method: Test principle
In the search for efficient insecticides, 33 different terpenoid compounds with a carbon skeleton similar to farnesol, a known insect juvenile hormone, were tested on four different insects for juvenile hormone activity.
Species
The insect species were Dysdercus cingulatus (Heteroptera, Pyrrhocoridae; red cotton bug), Tenebrio molitor (Coleoptera, Tenebrionidae; yellow mealworm), Musca domestica (Diptera, Muscidae; housefly) and Aedes aegypti (Diptera, Culicidae; yellow fever mosquito). Test setups for the different species were as follows.
D. cingulatus: Experiments were carried out on newly moulted larvae (0-20 h old) of the last, fifth stage.The insects were raised in glass jars at 24 °C and 70% RH. They were fed with cotton seeds.
T. molitor: Pupae (1-24 h after moulting) were used for investigation. The insects were raised in glass jas at 27 °C and 70-80% RH. The diet of the larvae contained bran, yeasts, flour and flaked oats.
M. domestica: Investigation of the efficiency of preparations studied were carried out on the last larval stages and early uncoloured stages of pupa ("white pupa"). The flies were kept at 27.5 °C. Larvae were fed withthe standard mixture "LSM" for laboratory mice and rats.
A. aegypti: The laboratory cultivation was carried out according to Byrdy (Pol Pismo Ent B 31-32: 129-151, 1963).
Application
Acetone solutions of the various test substances of various concentrations were applied topically by a droplet method in amounts of 1 ul on the cuticle of newly moulted larvae of D. cingulatus or pupae of T. molitor. The highest applied dose of

test substance was 80 ug per specimen, the control insects were treated with 1 ul of acetone.
The application of substances to larvae and pupae of M. domestica was performed in an analogous way with ehtanol as a solvent and at a maximal dose of 10 ug test substance per specimen.
In the case of A. aegypti, 0.2 ml of ethanol containing appropriate concentrations of test substance were added to 200 ml of food. The investigations on pupae of the thrid and fourth larval stages were carried out under these conditions. The control sample consisted of food with the addition of 0.2 ml of ethanol. The maximal concentration of test substance was 10 mg/l of food.
Evaluation
The biological activity was determined according to Slama et al. (Insect Hormones and Bioanalogues. No publisher given, Vienna & New York, 1974) by estimating the dose needed for 50% inhibition (ID50) of metamorphosis. This denotes the amount of substance in ug per compound and specimen that, when applied on the surface of newly moulted larvae or pupae, results in the formation of an intermediate (as opposed to fully metamorphous) form. In such an intermediate form, the front part of the body develops typically and becomes transformed into an adult form whereas the back part remains pupal in characteristics.

Result: Pseudoionone did not have any juvenile hormone activity in this test at the highest concentrations on D. cingulatus (80 ug/specimen) or on M. domestica (10 mg/specimen both for larvae and pupae). In contrast, pseudoionone showed an ID50 for metamorphosis at the highest concentration tested in T. molitor (80 ug/specimen). Moreover, pseudoionone was toxic to A. aegypti with an LC50 of 10.15 ug/l diet.
Within a group of six pseudoionone analogues, pseudoionone itself showed the lowest relative juvenile hormone activity, in some cases together with another compound, regarding T. molitor, D. cingulatus and M. domestica; it also had the lowest toxicity for A. aegypti. However, it is to be noted that not all congeners were tested for effects with all species. The authors conclude that within a group of pseudoionone analogues, those compounds with a straight alkyl chain (R = methanol, ethanol, n-propanol or n-butanol) in the 1-position exhibited increased activity, with the maximum occurring at R = n-propanol.
In comparison with other, non-pseudoionone-analogue compounds, pseudoionone showed both low relative inhibition of metamorphosis and low toxicity against A. aegypti.

Test substance: Test substances are described as "33 preparations with controlled purity, obtained by us earlier (Galera & Zabza, Bull Acad pol Sci, S Sci chim 25: 615-625, 1977; Galera & Zabza, Buall Acad pol Sci, S Sci chim 26: 427-439, 1978)". No further data available.

Conclusion: In a comparative study on four insect species, pseudoionone showed low juvenile hormone activity and low toxicity.

Reliability: (2) valid with restrictions
Old publication with short but clear methods, evaluation criteria and summary results, only the test substance is not characterised in detail. Judged to be of reliability 2.

Flag: Critical study for SIDS endpoint

02-DEC-2003 (62)

Species: other not soil dwelling arthropod: Tenebrio molitor (meal worm)
Endpoint: other: inhibition of development
Expos. period: 7 day(s)
Unit: other: µg/pupa, topical administration
NOEC: = 7.81 - measured/nominal
EC50 : = 62.5 - 125 measured/nominal
EC100 : = 250 - measured/nominal

Method: other: no data
Year: 1972
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: Pseudoionone in acetone as the solvent was applied topically to the venter of yellow meal worm (Tenebrio molitor) pupae of 48 hours age, as a single dose of 1000, 500, 250, 125, 62.5, 31.25, 15.62, 7.81 or 0 µg per pupa. Two 0-dose controls were run, acetone-only and no treatment at all. Twenty pupae per concentration were treated, the application volume per pupa was 5 µl. After application the pupae were kept singly in glass beakers in a temperature-controlled chamber (30 °C) at high humidity for 7 days. After 7 days, the number of normally metamorphosed mealworms and other pupae (no or only partial metamorphosis) was determined.

Result:

Pseudoionone dose, µg/pupa	% unaffected	% affected
0 (untreated controls)	100	0
0 (acetone controls)	89.5	10.5
7.81	100	0
15.62	79	21
31.25	85	15
62.5	65	35
125	6	94
250	0	100
500	0	100
1000	0	100

By crude visual interpolation on a log graph (not in reference) the EC50 corresponds to a dose of approximately 64 µg/pupa.
Test substance: Pseudoionone from Pfizer, no data on purity.
Conclusion: Pseudoionone has insect juvenile hormone activity and may therefore interrupt development of mealworms. At a topical dose between 62.5 and 125 µg/pupa it will inhibit metamorphosis in 50% of cases. The highest tested no-effect dose was 7.81 µg/pupa, the lowest tested 100%-inhibitory dose was 250 µg/pupa.
Reliability: (2) valid with restrictions
In spite of a lack of substance data, the test is clearly described, % results for all dose levels and controls are given and a conclusion is drawn. Reliability 2.
25-JUN-2003 (78)

Species: other not soil dwelling arthropod: Earias vittella (Lepidoptera, Noctuidae; noctuid moths)
Endpoint: other: inhibition of embryonic development
Expos. period: 96 hour(s)

Year: 1979

GLP: no data
Test substance: no data

Method: As several terpenoids have significant juvenile hormone activity, 16 terpenoids were tested for their effect on embryonic development in the noctuid moth, Earias vittellata. The test compounds were dissolved in isopropyl alcohol and spread in different concentrations on the bottom of glass tubes (2 cm diameter, 5 cm length). Based on listed results, the highest exposure concentrations corresponded to 133.2 ug/cm2.
Eggs of E. vittellata were arranged into groups of 20 and placed at different age levels (after egg deposition) in contact with the respective test compound in the glass tubes, kept at 27±1 °C and 60-70% RH and examined daily for the number of eggs hatched. Appropriate controls were run and each experiment was replicated five times.
The effect of terpenoids was expressed in term of per cent inhibition of embryonic development, normalised to hatching success in the controls.

Result: While several terpenoids inhibited partially or completely the embryonic development, pseudoionone at a probable highest concentration (not stated for pseudoionone but given as such for the active substances) of 133.2 ug/cm2 did not inhibit the embryonic development of Earias eggs of different ages.

Conclusion: Up to the highest concentration applied, probably 133.2 ug/cm2, pseudoionone was not inhibitory on egg development of a noctuid moth.

Reliability: (2) valid with restrictions
Short article, no characterisation of test substances and results only detailed for positive, inhibitory substances, but clear methods, sound approach and unambiguous conclusion for pseudoionone, hence reliabity tentatively set at 2.

24-JUN-2003 (68)

Species: other not soil dwelling arthropod: Pyrrhocoris apterus (Heteroptera, Pyrrhocoridae; fire bug)
Endpoint: other: inhibition of metamorphosis

Year: 1978
GLP: no data
Test substance: no data

Method: Fire bug, Pyrrhocoris apterus, larvae were used for this test. Experimental animals were kept on a disc of filter paper in an 11-cm Petri dish containing dry seeds and a cotton-plugged vial with water. Animals were kept at 27 °C with an 18-hour-light/6-hour-dark cycle.
Test substances including pseudoionone were either applied topically to P. apterus larvae by serially dissolving the test substance in acteone and applying 1 ml directly to the cuticle at doses of 0.1, 1 and 10 ug/specimen or applied indirectly by application of seriall solutions in acetone to the filter paper substrate at doses of 1, 10 and 50 ug/cm2 of filter paper.
For evaluation, the following parameters were recorded: 1) the length of the intercdysial period, indicating disturbances inthe moulting cycles; 2) qualitative changes in the succession of the larval instars, i.e. prothetely and metathely in technical terms; and 3) local prothetelies or

metathelies, indicating developmental disproportions between the different tissues of the larvae.

Result: Anti-ecdysone-like activity was noted for pure pseudoionone as an "antifeeding effect associated with reversible inhibition of larval development. The effects were characterised by suppressed or arrested feeding, though the food [itself] was not directly contaminated, decreased water uptake, prolonged interecdysial periods if ecdysis was at all evident, incomplete coordination of the locomotion and decreased survival. Specimens which overcame the ecdysial failures gave rise to extremely small adults with rudimentary wings."

Test substance: Test substances including pseudoionone "were received from the Department of Organic Chemistry, Harvard University". No further information on test substance in publication.

Conclusion: At unstated doses and possibly by direct and indirect application, pure pseudoionone had antiecdysone-like activity on larvae of the fire bug, P. apterus.

Reliability: (4) not assignable
Review article, secondary source, reliability 4.

02-DEC-2003 (89)

5.0 Toxicokinetics, Metabolism and Distribution

5.1 Acute Toxicity

5.1.1 Acute Oral Toxicity

Type: LD50
Species: rat
Strain: Wistar
Sex: male/female
No. of Animals: 10
Vehicle: other: 0.5% aqueous carboxymethyl cellulose
Doses: 2000 mg/kg bw
Value: > 2000 mg/kg bw

Method: other: not explicitly stated but corresponding to the EEC/OECD acute oral fixed-dose method
Year: 1988
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Animals and husbandry
Young Wistar rats were acquired from Dr. K. Thomae GmbH, Biberach, Germany, and acclimatised for at least 1 week before treatment. The animals were housed in fully air-conditioned rooms, with a temperature range of 20-24 °C, 30-70% relative humidity and a 12-hour-light (06:00-18:00)/12-hour-dark cycle. They had free access to rat diet (Kliba Labordiät 343, Klingenthalmühle AG, Kaiseraugst, Switzerland) and to tap water. The animals were randomised and kept in groups of 5 per sex in stainless-steel wire-mesh cages (Type DK-III, Becker & Co., Castrop-Rauxel, Germany). Groups were identified using cage cards. At the beginning of the test, the animals had a mean bodyweight of 189 g (5 males), respectively 180 g (5 females), with all animals being within ±20% of the mean bodyweight per sex.
Test substance formulation
Psudoionone was formulated with 0.5% aqueous carboxymethyl cellulose to give an emulsion. The concentration was selected based on the bodyweight of the test animals so that a dose volume of 10 ml/kg bw was attained. Only one concentration was tested, corresponding to 2000 mg/kg bw.
Dosing and observation
The test animals were fasted for about 16 hours before administration but had access to tap water throughout. The test article was administered by single oral gavage dosing in the morning of test day 1, 13-Sep-1988. The test animals were replaced in their respective cage and kept for a post-dosing period of 14 days. During this period they were observed for signs and symptoms severely times on the day of administration and at least once each workday. Checking for moribund or dead animals was done twice each workday and once on weekend days.
Killing and necropsy
After 14 days' observation period, feed was withheld for about 16 hours before carbon dioxide asphyxiation, which was followed by weighing and necropsy with gross-pathological examination.

Statistics and data archiving
As there were no deaths during the study, no statistical evaluation was performed. All raw data, study documents and the report are kept at BASF AG, Ludwigshafen, Germany.

Result: There were no deaths during the study. There were no unusual signs or symptoms recorded for the males or for the females. Males showed a bodyweight gain from an average of 189 g on day 1 to an average of 295 g on day 13 before killing; females showed a bodyweight gain from an average of 180 g on day 1 to an average of 221 g on day 13. On necropsy there were no pathological signs noted.

Test substance: Pseudoionone from BASF AG. "A detailed product characterization is included in the raw data" (Test report, page 1).

Conclusion: The oral (gavage) LD50 for rats was > 2000 mg pseudoionone/kg bw, for both males and females. The administered dose of 2000 mg/kg bw was both the LD0 and the NOEL.

Reliability: (2) valid with restrictions
Short but detailed professional report from an industry toxicity laboratory. The only information that is missing is the precise test guideline followed and the certificate of analysis for the test substance sample, the latter is noted to be kept with the raw data in the archive. Reliability 2.

Flag: Critical study for SIDS endpoint

22-JAN-2003 (57)

Type: LD50
Species: rat
Strain: other: Roche inbred strain
Sex: no data
Vehicle: no data
Doses: 1000, 2000, 4000 and 8000 mg/kg bw
Value: > 8000 mg/kg bw

Method: other: gavage oral toxicity, F. Hoffmann-La Roche test
Year: 1973
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: As usual for this internal Roche testing scheme, groups of 5 or 10 animals per dosage were used. Administration was by gavage. Observation was 10 days after administration, then the test animals were killed and dissected. Statistics were computed if applicable. Controls were historical with the same rat strains.

Result:

Pseudoionone, daily dose, mg/kg bw	Deaths at 24 hours after administration	Deaths at 10 days after administration
8000	0	0
4000	0	0
2000	0	0
1000	0	0

LD0 = 8000 mg/kg bw
LD50 > 8000 mg/kg bw

Test substance: Pseudoionone, Roche, Lot Mag-No 3003.
Conclusion: Pseudoionone is of low acute toxicity.
Reliability: (2) valid with restrictions
While this test is reported only in very abbreviated form, the acute toxicity group led by the author of the report performed large series of highly standardised toxicity tests

in the late 1960s, 1970s and early 1980s. Serial testing in a dedicated facility assures dependably regular animal keeping, test substance administration, laboratory protocol and reporting. Therefore these internal data are regarded as valid and dependable.

Flag: Critical study for SIDS endpoint

10-JAN-2006 (12)

Type: LD50
Species: mouse
Strain: other: Roche inbred strain
Sex: no data
Vehicle: no data
Doses: 1000, 2000, 4000 and 8000 mg/kg bw
Value: = 7270 mg/kg bw

Method: other: Roche gavage oral toxicity test
Year: 1973
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: As usual for this internal Roche testing scheme, groups of 5 or 10 animals per dosage were used. Administration was by gavage, once daily for five consecutive days. Observation was 1 day after administration, then the test animals were killed and dissected. Statistics were computed if applicable. Controls were historical with the same mouse strains.

Result:

Pseudoionone, daily dose, mg/kg bw	Deaths, %, on day 1
8000	80
4000	0
2000	0
1000	0

Based on the deaths noted, the following oral lethal dose values in mg/kg bw were interpolated:

	24 h post single dose
LD10	4750
LD50	7270±1100
LD90	>8000

Test substance: Pseudoionone, Roche, Lot Mag-No 3003.
Conclusion: Pseudoionone is of low acute toxicity.
Reliability: (2) valid with restrictions
While this test is reported only in very abbreviated form, the acute toxicity group led by the author of the report performed large series of highly standardised toxicity tests in the late 1960s, 1970s and early 1980s. Serial testing in a dedicated facility assures dependably regular animal keeping, test substance administration, laboratory protocol and reporting. Therefore these internal data are regarded as valid and dependable.

23-MAR-2004 (13)

Type: LD50
Species: mouse
Strain: NMRI
Sex: male/female
No. of Animals: 16
Vehicle: other: maize/corn oil
Doses: 0 (vehicle control), 2000 mg/kg bw

Value: >= 2000 mg/kg bw

Method: other: OECD 473
Year: 2003
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: In an in vivo micronucleus mutagenicity test according to OECD 473, NMRI BR (SPF) mice from Charles River, Sulzfeld, Germany were used. Animals were young adults (6-8 weeks old), females were nulliparous and non-pregnant.Full details reagrding animal source, housing, and keeping as well as test procedures are given in chapter 5.6.
For the dose range-finding test, 3 males and 3 females were used. They were given a single dose of 2000 mg pseudoionone/kg bw dissolved in maize/corn oil at a dose volume of 10 ml/kg bw. All 6 range-finder animals survived for three days, hence a dose of 2000 mg/kg bw was selected for the main test.
In the main test there were 4 groups, labelled A through D. A was a negative control (vehicle only, 10 ml maize/corn oil/kg bw) group, B and C were treatment groups (5 animals, each, males only, 2000 mg pseudoionone/kg bw in maize/corn oil, dose adjusted to a volume of 10 ml/kg bw; group B to be sampled at 24 hours post-dosing, group C at 48 hours post-dosing) and D was a positive control group (50 mg cyclophosphamide/kg bw, dissolved in physiological saline; cyclophosphamide from Asta-Werke, Germany). Feed was withheld 3-4 hours prior to dosing. Administration was by oral gastric intubation.
Observations
The animals were observed at least once a day for signs of toxicity. Prior to dosing the animals were weighed.
Result: At a single oral dose by gavae of 2000 mg pseudoionone/kg bw, all 3 males and 3 females in the range-finder survived the test duration of 72 hours, as did the 5 and 5 males in the main test groups for a duration of 24 or 48 hours, respectively. No toxic effects were noted during regular observations.
Test substance: Pseudoionone from Teranol AG, Lalden, Switzerland, lot no. UU02033826, purity 95.4% area, GC), complying with specification. Certificate of analysis no. 554, dated 28-MAR-2002, Quality Control Department, Teranol, Lalden
Conclusion: In confirmation of the actual acute oral toxicity tests, all of 16 mice (3 females and 3 males in the range-finding test, 5 and 5 males in the two test groups in the main test) survived an oral dose of 2000 mg/kg bw in maize/corn oil for at least 24 up to 72 hours. No toxic effects were noted during regular observations.
Reliability: (2) valid with restrictions
Not an actual acute toxicity test but a pretest and main test under GLP according to an OECD guideline with full details in report. Reliability 2.
15-JAN-2004 (10)

Type: LD50
Species: rat
Strain: no data
Sex: no data
No. of Animals: 10
Vehicle: no data

Doses: at least 5000 mg/kg bw
Value: > 5000 mg/kg bw

Method: other: no data
Year: 1976
GLP: no data
Test substance: no data

Result: Based on 0 out of 10 rats dead after application of 5000 mg pseudoionone/kg bw, the LD50 is > 5000 mg/kg bw and the NOEL is = 5000 mg/kg bw.
Conclusion: Pseudoionone is of low acute oral toxicity with an oral NOEL of 5000 mg/kg bw and an acute oral LD50 > 5000 mg/kg bw.
Reliability: (2) valid with restrictions
While only the short abstract was seen, which does not list any details, the test was performed by a professional toxicologist who screened many flavour and fragrance substances and related compounds for RIFM-FEMA, hence reliability was set 2.
15-JAN-2004 (70)

5.1.2 Acute Inhalation Toxicity

5.1.3 Acute Dermal Toxicity

Type: LD50
Species: rabbit
Strain: no data
Sex: no data
No. of Animals: 10
Vehicle: no data
Doses: at least 5000 mg/kg bw
Value: > 5000 mg/kg bw

Year: 1976
GLP: no data
Test substance: no data

Method: Application
Ten healthy albino rabbits received one dermal application of test material. The test material was applied to clipped, intact or abraded abdominal skin under occluded patches for 24 hours of contact.
Observation
Observations for mortality and/or systemic effects were made daily for 14 days following application. Dermal reactions were scored on days 1, 7 and 14 after application using the Draize scoring system. On day 14 after application, test animals were killed and gross necropsy was performed on all animals.
(cited from the RIFM-FEMA Database entry)
Result: Based on 1 out of 10 animals dead after apllication of 5000 mg/kg bw, the dermal LD50 is given as >5000 mg/kg bw.
Conclusion: Pseudoionone has a low acute dermal tosicity with a dermal LD50 > 5000 mg/kg bw.
Reliability: (2) valid with restrictions
While only the short abstract was seen, which does not list

any details, the test was performed by a professional toxicologist who screened many flavour and fragrance substances and related compounds for RIFM-FEMA, hence reliability was set 2.

Flag: Critical study for SIDS endpoint

15-JAN-2004 (70)

5.1.4 Acute Toxicity, other Routes

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: 2.9
Result: irritating
EC classificat.: irritating

Method: OECD Guide-line 404 "Acute Dermal Irritation/Corrosion"
Year: 1990
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Animals and husbandry
White Vienna rabbits were obtained from Gaukler (Offenbach, Germany) The animals were acclimatised at least 8 days before the beginning of studies and kept singly in stainless-steel cages with wiremesh floor. There was no bedding in the cages but sawdust in the waste trays. The animals were housed in fully air-conditioned rooms, with a temperature range of 20-24 °C, 30-70% relative humidity and a 12-hour-light (06:00-18:00)/12-hour-dark cycle. They had free access to rabbit diet (Kliba 341, 4 mm, Klingenthalmühle AG, Kaiseraugst, Switzerland) and approximately 250 ml of tap water per day. Animals were identified by unique ear tattoo.
Test procedure
For the pseudoionone test, two males (internal no. 0612, 2.69 kg bw; internal no. 0608, 2.40 kg bw) and one female (internal no. 0650, 2.48 kg bw) were used. At least 15 hours before application of the test substance, the fur was clipped on the dorsum of the rabbits. A volume of 0.5 ml undiluted pseudoionone was applied to patches of 2.5x2.5 cm, one patch each was applied to the upper third of the flank or back one one side of each animal, the other side serving as a negative control. Patches were secured in position with a porous dressing consisting of four layers of absorbent gauze and porous bandage. After 4 hours' exposure, the patches were removed and remaining test substance was washed off with a 1:1 mixture of Lutrol and water. The first reading of skin reactions was made 30-60 minutes after removal of the patches and at 24, 48 and 72 hours as well as at 8 and 15 days after start of application. Total duration of the test including

observation was 15 days. Skin reactions (erythema and/or oedema) were graded by a veterinarian according to EEC criteria as follows: 0 = none, 1 = very slight, 2 = well-defined, 3 = moderate to severe, 4 = severe to very severe. For the calculation of mean erythema and oedema only the readings at 24, 48 and 72 hours were used.

Result: The following readings were taken:

Time after appl.	Animal no. (sex)		
	1 (m)	2 (m)	3 (f)
4 hours	E2,O1	E2,O2	E2,O1
24 hours	E3,O2	E3,O3e	E3,O1
48 hours	E3,O2	E3,O3e	E3,O0
72 hours	E3,O1	E3,O1	E2,O0
8 days	E2,O0s	E1,O0s	E1,O0s
15 days	E2,O1s	E1,O0s	E1,O0s

E = erythema
O = oedema
e = extended erythema
s = scaling

Average values per animal (24, 48 and 72 hours)

Erythema	3.0	3.0	2.7
Oedema	1.7	2.3	0.3

Average values for all 3 animals (24, 48 and 72 hours)

Erythema	2.9
Oedema	1.4

Test substance: Pseudoionone from BASF AG. "A detailed product characterization is included in the raw data" (Test report, page 1).

Conclusion: Undiluted pseudoionone applied to rabbit skin under semi-occlusive conditions resulted in moderate to severe erythema, with an average primary irritation index of 2.9 for the first 72 hours, that was slow to resolve, with very slight to well-defined erythema remaining after 15 days. Oedematous reactions were weakerbut also pronounced at 48 and 72 hours. Pseudoionone is a skin irritant.

Reliability: (2) valid with restrictions
Short but detailed professional report from an industry toxicity laboratory with all single readings given. The only information that is missing is the certificate of analysis for the test substance sample, which is noted to be kept with the raw data in the archive. Reliability 2.

Flag: Critical study for SIDS endpoint

06-JAN-2003 (43)

Species: guinea pig
Exposure: Occlusive
Exposure Time: 24 hour(s)
Vehicle: water

Method: other: OECD Guideline 406
Year: 1996
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: A main skin sensitisation study using 20 test and 10 control guinea pigs was initiated using concentrations of 5% v/v pseudoionone in water for the intradermal induction and 50% v/v in water for the topical induction. However, following the topical induction phase the behaviour and general condition of the animals indicated that a severe skin response had

occurred. The sensitisation study was therefore aborted. Data from this aborted study have not been reported but remain archived in the contract laboratory.
The potential of topical pseudoionone to cause skin irritation was therfore re-assessed by means of a topical concentration ranging study using 4 guinea pigs that were in the weight range of 379-431 g and which had been previously treated with 4 intradermal injections of a 1:1 mixture of Freund's Complete Adjuvant (FCA) and water. The concentrations used were 100%, 50%, 25% and 12.5% pseudoionone as a suspension in water. An area of 8x5 cm was clipped free of fur over the back and flanks of 4 animals and 4 patches of Whatman No 3 filter paper, 2 cm x 2 cm in size, each saturated with a different concentration of the test article in water were placed on the skin, 2 patches on each flank. A strip of 5-cm-wide 'Blenderm' surgical tape was places over the patches to act as an occlusive barrier and the patches held in place for 24 hours by encircling the trunk of each animal with 'Elastoplast' ealstic adhesive bandage.
24 and 48 hours after removing the patches and dressings the animals were examined under a standard light source designed to comply with the requirements of BS (British Standard) 950 Part 1, Artificial daylight for the assessment of colour.
Responses were assessed and scored using the following system:

Skin reaction	Score
No visible reaction	0
Discrete or patchy erythema	1
Moderate or confluent erythema	2
Intense erythema and swelling	3

On all four animals pseudoionone caused intense brown staining an most test sites, preventing a full assessment of the skin responses. The incidence of obscured sites was related to the test article concentration. Based on this initial result, pseudoionone concentrations of 6.25%,3.125%, 1.563% and 0.78% v/v in water were applied to skin sites prepared as above on a fifth, non-pretreated animal weighing 502 g in an attempt to determine the highest pseudoionone concentration that would not stain the skin to a degree which prevented assessment of the resulting skin response.

Result: In the first skin sensitisation main study a topical second induction using 50% pseudoionone resulted in "severe" skin responses as evidenced by behaviour and general condition of the animals. This study was then aborted.
In the subsequent skin irritation ranging study, topical pseudoionone caused intense brown staining at most test sites in all four first animals, preventing a full assessment of the skin responses. The incidence of obscured sites was related to the test article concentration. Based on a fifth animal with lower concentrations as described above, the results of the preliminary range finder indicated that 6.25% pseudoionone in water was the highest concentration to produce moderate irritation in the short term, which would resolve within a few days. In the subsequent main test, however, it was shown that the staining reactions subsequent to topical challenge with 6.25% pseudoionone in water were still so strong that reading and grading of skin reactions were impossible. A second challenge with 3.125% and 1.563% pseudoionone in water resulted in weak staining that did not, however, preclude observation. None of the animals in this second challenge

showed any skin responses.
Test substance: Pseudoionone from Teranol AG, Lalden, Lot no. 05076, Analysis no. 575E6, purity 91.2% (area, GC).
Conclusion: A concentration of 50% pseudoionone suspended in water, applied by occlusive application to the skin over 24 hours, resulted in "severe" skin reactions. In a further topical challenge, 3.125% pseudoionone suspended in water (and lower concentrations) did not elicit any irritant reactions; the reaction to 6.25% could not be scored because of staining of the skin. Higher concentrations (10% and more) in range-finding tests resulted in severe irritation up to necrosis.
Reliability: (2) valid with restrictions
First (later aborted) OECD GLP skin sensitisation test and subsequent irritation range-finding study. The single observations of the first test and of the range finder are both given in the full test report. Even though this was a sensitisation study, all data are given and so are the clear conditions for scoring effects, hence reliability is set at 2.

22-JAN-2003 (20)

Species: guinea pig
Concentration: 5 %
No. of Animals: 1
Vehicle: water

Method: other: OECD Guideline 406
Year: 1996
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: In the course of a GLP skin sensitisation test an intradermal injection concentration ranging study was performed in one guinea pig before the start of the main study, to determine a suitable concentration of the test article for the intradermal injection stage of the main study. This ranging study was performed in one animal which, 7 days before, had been pretreated with 4 intradermal injections of a 1:1 mixture of Freund's Complete Adjuvant (FCA) and water. Then, 0.1-ml aliquots of 50%, 25%, 10%, 5%, 1% and 0.5% v/v concentrations of the test article in water were injected intradermally into the flanks of the guinea pig. The animal was examined on the day of dosing and then daily for a further 5 days and the response at each injection site was noted.
Result: From the results of this range finder it was concluded that 5%, 1% and 0.5% v/v concentrations of pseudoionone in water administered subcutaneously would provoke only a moderate irritant response, as evidenced by 5-mm erythemata on all three sites on day 2, all three of which narrowed to 3 mm diameter on days 5 and 6. The higher concentrations (10%, 25% and 50%) did cause lasting and more severe irritant responses as shown by formation of white foci that became bigger over time, surrounded by erythema. Also, the main test did result in moderate irritation at the injection site in the short term, which later resolved.
Test substance: Pseudoionone from Teranol AG, Lalden, Lot no. 05076, Analysis no. 575E6, purity 91.2% (area, GC).
Conclusion: An intradermal injection of 0.1 ml of 0.5% to 5% pseudoionone in water was moderately irritating to guinea pig skin. Higher

concentrations caused clear irritation or growing white foci that are interpreted as tissue necrosis. Intradermal pseudoionone is judged to be moderately to strongly irritating to skin, depending on concentration.

Reliability: (2) valid with restrictions
Intradermal range-finding study to and the intradermal induction part of an OECD GLP skin sensitisation test. Even though this was a sensitisation study, all data are given and so are the clear conditions for scoring effects, hence reliability is set at 2.

11-FEB-2003 (20)

Species: rabbit
Concentration: undiluted
Exposure: Occlusive
Exposure Time: 24 hour(s)
No. of Animals: 10
Vehicle: other: no vehicle
Result: moderately irritating

Year: 1976
GLP: no
Test substance: no data

Result: In the acute dermal toxicity test [Moreno, 1976; cited in Ford et al., 1988], undiluted pseudoionone was applied to the skin of 10 rabbits using occlusive patches for 24 hours and dermal reactions were scored on days 1, 7 and 14. In the short report, pseudoionone is stated to have "produced moderate irritant effects", which, however, were not otherwise described nor were any scores reported.

Conclusion: In an acute dermal toxicity test, undiluted pseudoionone applied under occlusion for 24 h at a dose of 5000 mg/kg bw produced moderate irritant effects.

Reliability: (2) valid with restrictions
While only the short abstract was seen, which does not list any details, the test was performed by a professional toxicologist who screened many flavour and fragrance substances and related compounds for RIFM-FEMA, hence reliability was set 2.

15-JAN-2004 (70)

Species: human
Concentration: 8 %
Exposure: Occlusive
Exposure Time: 48 hour(s)
No. of Animals: 108
Vehicle: petrolatum
Result: not irritating

Method: other
Year: 1976
GLP: no
Test substance: no data

Result: In the Fragrance Raw Materials Monograph for pseudoionone, Ford and colleagues cite two unpublished studies performed on behalf of the Research Institute for Fragrance Materials by AM Kligman (1976) and WL Epstein (1978):
"A 48-hr closed-patch test at a concentration of 8% in

petrolatum on the forearms or backs of 108 volunteers produced no irritation (Epstein, 1978; Kligman, 1976)."

Conclusion: Based on a secondary source, pseudoionone is not irritating to human skin in a concentration of 8%.

Reliability: (2) valid with restrictions
Secondary source, reliability 4.

15-JAN-2004 (34)

5.2.2 Eye Irritation

Species: rabbit
Concentration: undiluted
Dose: .1 ml
Exposure Time: unspecified
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: 1990
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Animals and husbandry
White Vienna rabbits were obtained from Gaukler (Offenbach, Germany). The animals were acclimatised at least 8 days before the beginning of studies and kept singly in stainless-steel cages with wiremesh floor. There was no bedding in the cages but sawdust in the waste trays. The animals were housed in fully air-conditioned rooms, with a temperature range of 20-24 °C, 30-70% relative humidity and a 12-hour-light (06:00-18:00)/12-hour-dark cycle. They had free access to rabbit diet (Kliba 341, 4 mm, Klingenthalmühle AG, Kaiseraugst, Switzerland) and approximately 250 ml of tap water per day. Animals were identified by unique ear tattoo.
Test procedure
For the pseudoionone test, one male (internal no. 0613, 2.73 kg bw) and two females (internal no. 0582, 3.13 kg bw; internal no. 0652, 2.83 kg bw) were used. A single application of 0.1 ml undiluted pseudoionone into the conjunctival sac of the right eyelid was made. The substance was not washed out by rinsing. The untreated controlateral eye served as the control. The total observation period was 8 days, readings were taken at 1, 24, 48, 72 and 192 hours after application. Grading of reactions was taken according to the OECD Guideline scale.

Result: The following readings were taken:

Time after appl.	Animal no. (sex)		
	1 (f)	2 (m)	3 (f)
1 hour	CO0,CA0,IR0	CO0,CA0,IR0	CO0,CA0,IR0
	CR2,CC0,DI1	CR2,CC1,DI2	CR2,CC1,DI2
24 hours	CO1,CA1,IR0	CO0,CA0,IR0	CO0,CA0,IR0
	CR2,CC1,DI1	CR2,CC1,DI2	CR2,CC1,DI0
48 hours	CO0,CA0,IR0	CO0,CA0,IR0	CO0,CA0,IR0
	CR2,CC1,DI0	CR1,CC0,DI0	CR2,CC0,DI0
72 hours	CO0,CA0,IR0	CO0,CA0,IR0	CO0,CA0,IR0

	CR2,CC0,DI0	CR0,CC0,DI0	CR2,CC0,DI0
8 days	CO0,CA0,IR0	CO0,CA0,IR0	CO0,CA0,IR0
	CR0,CC0,DI0	CR0,CC0,DI0	CR0,CC0,DI0

CO = corneal opacity
CA = corneal area involved
IR = iris
CR = conjunctival redness
CC = conjunctival chymosis
DI = Discharge

Average values per animal (24, 48 and 72 hours)

CO	0.3	0.0	0.0
IR	0.0	0.0	0.0
CR	2.0	1.0	2.0
CC	0.7	0.3	0.3

Average values for all animals (24, 48 and 72 hours)

CO	0.1
IR	0.0
CR	1.7
CC	0.4

Test substance: Pseudoionone from BASF AG. "A detailed product characterization is included in the raw data" (Test report, page 1).

Conclusion: The administration of 0.1 ml undiluted pseudoionone caused mainly well-defined conjunctival reddening and transient slight chymosis. Most findings had resolved within 72 hours, none were detected after 8 days. Pseudoionone is slightly irritating to the eye but without long-term damage or effects.

Reliability: (2) valid with restrictions
Short but detailed professional report from an industry toxicity laboratory with all single readings given. The only information that is missing is the certificate of analysis for the test substance sample, which is noted to be kept with the raw data in the archive. Reliability 2.

Flag: Critical study for SIDS endpoint

11-FEB-2003 (44)

Species: rabbit
Result: slightly irritating
EC classificat.: not irritating

Method: OECD Guide-line 405 "Acute Eye Irritation/Corrosion"
GLP: no data

Result: In a broad German validation study, two in vitro alternative tests to the Draize eye irritation test, viz. the Hen's Egg Chorio-Allantoic Membrane (HET-CAM) test according to Luepke (Fd Chem Toxicol 23: 287-291, 1985) and the mammalian 3T3 cell line toxicity test with Neutral Red Uptake (3T3 NRU test) according to Borenfreund and Puerner (Toxicol Lett 24: 119-124, 1985), were compared with conventional Draize test results.
Draize tests had previously been performed by "chemical and pharmaceutical companies" according to OECD Guideline 405. No further details for single tests are available. Draize test data for 2-pseudoionone are listed as follows:

Time h	Conjunctival erythema	Conjunctival chemosis	Iris score	Corneal opacity
1	2.0	0.7	0.0	0.0
24	2.0	1.0	0.0	0.3

	48	1.7	0.3	0.0	0.0
	72	1.3	0.0	0.0	0.0
	mean	1.7	0.4	0.0	0.1

Test substance: "2-Pseudoionone", not otherwise specified in secondary source, no CAS number given.

Conclusion: Spielmann and colleagues report a Draize test according to OECD 405 from industry. As the overall scores are identical to the above industry test by Hildebrand and Kirsch [1990], in spite of a slightly different format given, it is concluded that Spielmann and co-workers report the above test.
In a reported Draize test from industry, 2-pseudoionone was slightly irritating as evidenced by a mean conjunctival erythema score of 1.7. According to EU classification criteria, this score does not entail a classification as irritating to the eye.

Reliability: (4) not assignable
This publication summarises many industry test results. While there is some level of detail and while the original tests may well be highly reliable, the available publication is a secondary source that does not give detailed methods and readings for single animals. In view of the high probability that the present test is a secondary source for the earlier report by Hildebrand and Kirsch [1990], the reliability was set at 4.

15-JAN-2004 (91)

Species: other: alternative in vitro tests: HET-CAM and 3T3 NRU
Vehicle: no data
Result: slightly irritating
EC classificat.: not irritating

Method: other: HET-CAM and 3T3 NRU in vitro tests
GLP: no data
Result: In a broad German validation study, two in vitro alternative tests to the Draize eye irritation test, viz. the Hen's Egg Chorio-Allantoic Membrane (HET-CAM) test according to Luepke (Fd Chem Toxicol 23: 287-291, 1985) and the mammalian 3T3 cell line toxicity test with Neutral Red Uptake (3T3 NRU test) according to Borenfreund and Puerner (Toxicol Lett 24: 119-124, 1985), were compared with conventional Draize test results.
In the Appendix, 2-pseudoionone is characterised as not labelled according to EC criteria, meaning not irritant to the eye, both by Draize test and by in vitro studies.
Specifically, 2-pseudoionone is listed to have an irritation threshold >100%, meaning no irritation, in the HET-CAM test according to data supplied by Henkel KgaA, Germany and the German Bundesgesundheitsamt (Health Office). In the 3T3 NRU test, the IC50 for 2-pseudoionone was 0.08 mg/ml in the Henkel test and 0.04 mg/ml in the Bundesgesundheitsamt test.

Test substance: "2-Pseudoionone", not otherwise specified in secondary source, no CAS number given.

Conclusion: 2-Pseudoionone did not test positive in two in vitro alternative tests to the Draize eye irritation test in a broad German validation study.

Reliability: (4) not assignable
Secondary source, no test details, only summary results, reliability 4.

17-NOV-2003 (91)

5.3 Sensitization

Type: Guinea pig maximization test
Species: guinea pig
Concentration 1st: Induction 50 % intracutaneous
2nd: Induction 12.5 % occlusive epicutaneous
3rd: Challenge 6.25 % occlusive epicutaneous
No. of Animals: 30
Vehicle: other: suspension in water or emulsion with Freund's Complete Adjuvant
Result: not sensitizing
Classification: not sensitizing

Method: OECD Guide-line 406 "Skin Sensitization"
Year: 1996
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: Animals, husbandry and diet
Young female nulliparous, non-pregnant albino guinea pigs of Dunkin Hartley strain were supplied by D Hall (Burton-on-Trent, England). The animals were ordered in the weight range 300-350 g and were delivered on 16-Aug-1996. Additional animals for the main study were delivered on 20-Sep-1996. Both for the range-finding study and for the main study, the animals were acclimatised for 5 days.
Animals were housed in groups of up to 5 in stainless-steel cages and identified by number of cage and ear tattoo.
SQC FD1 pelleted guinea pig diet with added vitamin C (Special Diets Services, Witham, England) and mains drinking water were freely available. Certificates of analysis for both diet and drinking water are held on file at the test laboratory.
The animal room was air-conditioned with temperature within the range of 20-23 °C and relative humidity within the range of 36-68% during both acclimatisation and study periods. Fluorescent lighting gave an artificial cycle of 12 hours light (06:00-18:00) and 12 hours dark per day.
Intradermal injection concentration ranging study
Before the start of the main study, an injection concentration range-finding study was performed to determine a suitable concentration of the test article for the intradermal injection stage of the main study. This ranging study was performed in one animal which, 7 days before, had been pretreated with 4 intradermal injections of a 1:1 mixture of Freund's Complete Adjuvant (FCA) and water. Then, 0.1-ml aliquots of 50%, 25%, 10%, 5%, 1% and 0.5% v/v concentrations of the test article in water were injected intradermally into the flanks of the guinea pig. The animal was examined on the day of dosing and then daily for a further 5 days and the response at each injection site was noted. From the results of this range finder it was concluded that a 5% v/v concentration of pseudoionone in water would not provoke an unacceptable irritant response and this concentration was therefore selected for use in the intradermal injection phase of the study.
Topical irritancy ranging study
The potential of the test article to cause skin irritation was assessed by means of a topical concentration ranging study using 4 animals that were in the weight range of 379-431 g and which had been previously treated with 1:1 FCA/water as

described above. The concentrations used were 100%, 50%, 25% and 12.5% pseudoionone as a suspension in water. An area of 8x5 cm was clipped free of fur over the back and flanks of 4 animals and 4 patches of Whatman No 3 filter paper, 2x2 cm in size, each saturated with a different concentration of the test article in water were placed on the skin, 2 patches on each flank. A strip of 5-cm-wide 'Blenderm' surgical tape was places over the patches to act as an occlusive barrier and the patches held in place for 24 hours by encircling the trunk of each animal with 'Elastoplast' elastic adhesive bandage. 24 and 48 hours after removing the patches and dressings the animals were examined under a standard light source designed to comply with the requirements of BS (British Standard) 950 Part 1, Artificial daylight for the assessment of colour. Responses were assessed and scored using the following system:

Skin reaction	Score
No visible reaction	0
Discrete or patchy erythema	1
Moderate or confluent erythema	2
Intense erythema and swelling	3

On all four animals pseudoionone caused intense brown staining on most test sites, preventing a full assessment of the skin responses. The incidence of obscured sites was related to the test article concentration. Based on this initial result, pseudoionone concentrations of 6.25%,3.125%, 1.563% and 0.78% v/v in water were applied to skin sites prepared as above on a fifth animal weighing 502 g in an attempt to determine the highest pseudoionone concentration that would not stain the skin to a degree which prevented assessment of the resultung skin response. The results of the preliminary range finder indicated that 6.25% pseudoionone in water was the highest non-irritant concentration which could be applied to the skin without obscuring the site through intense staining and this concentration was therefore selected for the challenge phase. Additionally, a concentration of 12.5% v/v in water was selected for the topical induction phase of the main study.

Main study induction
A main study using 20 test and 10 control animals was initiated using concentrations of 5% v/v pseudoionone in water for the intradermal induction and 50% v/v in water for the topical induction. However, following the topical induction phase the behaviour and general condition of the animals indicated that a severe skin response had occurred. The study was therefore aborted and the main study resumed using untreated animals and a lower concentration for the topical induction phase. Data from the aborted study have not been reported but remain archived with the data from the replacement study that are detailed below.
Definitive main study: first, intradermal induction. 30 healthy animals were selected for the study and randomly allocated to a group of 20 test animals and a group of 10 control animals using a stratified bodyweight procedure. All were within the weight range of 341-389 g on day 1. The dorsal area between the shoulders of each animal was clipped free of fur and 3 pairs of intradermal injections were made within this area. The dose volume of each injection was 0.1 ml and each pair of injections consisted of:
a) Test group: 1) 50% v/v FCA emulsified with water
2) 5% v/v pseudoionone in water

3) 5% v/v pseudoionone in 1:1 FCA:water
b) Controls: 1) 50% v/v FCA emulsified with water
2) 100% water
3) 50% v/v water in 1:1 FCA:water.
Twenty-four hours after administration of the intradermal injections, all animals were examined for signs of irritation in the treated area.
Definitive main study: second, topical induction. Six days after intradermal induction, the area surrounding the injection sites of all test and control animals was again clipped free of fur and painted with 0.5 ml of 10% w/v sodium lauryl sulfate in light liquid paraffin. The following day, patches of Whatman No 3 filter paper, 4x2 cm, each saturated with 12.5% v/v pseudoionone in water, were placed over the injection sites of all animals in the test group in order to boost the induction process. A strip of 5-cm-wide 'Blenderm' was placed over the patch to act as an occlusive barrier and the whole assembly held in place by wrapping the trunk of each animal with a length of 'Elastoplast'. Animals of the control group were similarly treated, the patch of filter paper being saturated with water. The patches and dressings were removed after 48 hours. A further 24 hours after removal of the patches all animals were re-examined for signs of irritation in the treated area.
Main study: topical challenges. 14 days after the topical induction application, the fur was clipped free of fur and patches of Whatman No 3 filter paper, 2x2 cm, each saturated with 6.25% v/v pseudoionone in water, were placed on the left flank of all test and control animals. The right flank of each test and control animal was similarly treated with a patch soaked with water alone. The patches were occluded and secured using the method described above. After a contact period of 24 hours the dressings and patches were removed. After a further 24 and 48 hours the treated sites of all animals were examined for reaction to treatment. In the majority of animals, it was still not possible to score the reaction using the above scale because of skin staining that obscured all potential reactions. Therefore, a re-challenge was conducted 7 days later under the same conditions as the initial challenge, using pseudoionone concentrations of 3.125% and 1.563% in water. As in the initial challenge, reactions were scored 24 and 48 hours after removal of dressings and patches.

Result: 24 hours after challenge patch removal, the sites on 19 test animals an 6 controls treated with 6.25% v/v pseudoionone in water could not be assessed for reaction to treatment because of intense red-brown skin staining. 48 hours after the end of the occlusion period, the treated sites on 14 test and 5 control animals could still not be assessed. All sites treated with the vehicle, water, on all animals were assessed and scored no positive responses at either 24 or 48 hours after removal of occlusive patches.
Following re-challenge with 3.125% and 1.563% v/v pseudoionone in water, slight brown staining was apparent on the treated sites on most animals but this did not prevent the assessment of skin reactions. None of the animals in the test or control groups responded positively to either test article concentration at 24 or 48 hours observation.

Test substance: Pseudoionone from Teranol AG, Lalden, Lot no. 05076, Analysis no. 575E6, purity 91.2% (area, GC).

Conclusion: Pseudoionone is not a skin sensitiser.

Reliability: (1) valid without restriction
Reliability is set at 1 based on OECD protocol, GLP and detailed test report with data for every single animal in all pretests/range finders and main tests.
Flag: Critical study for SIDS endpoint
15-JAN-2004 (20)

Type: Patch-Test
Species: other: human volunteers
Concentration 1st: 8 %
No. of Animals: 108
Vehicle: petrolatum
Result: sensitizing
Classification: sensitizing

Method: other: Maximisation test
Year: 1976
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Remark: Based on these data, both the International Fragrance Association (IFRA) and subsequently the European Union Scientific Committee on Cosmetic Products and Non-Food Products (SCCNFP) recommended a ban of the use of pseudoionone as a fragrance ingredient.

Result: In the RIFM Monograph for pseudoionone, Ford and colleagues [1988] cite 4 maximisation test series performed by Kligman (1976, 2 series) and Epstein (1978, 2 series) with a total of 108 volunteers. As a result, "2/25 (Kligman, 1976), 4/25 (Epstein, 1978), 2/25 (Kligman, 1976) and 1/33 (Epstein, 1978) sensitization reactions" were produced, without further details as to the reactions. This corresponds to a total incidence of 9 positives out of 108 subjects or 8.3%. No further details can be derived from the publication and the original reports were never published, hence there is no information on possible earlier exposure of the probands to pseudoionone or similar substances.
Reliability: (2) valid with restrictions
While details as to the test series are lacking, the source is one of many monographs on fragrance compounds that have been compiled by the Research Institute for Fragrance Materials (RIFM) in the USA. The original reports are from highly experienced and respected dermal toxicologists who tested many fragrance and flavour compounds and related substances for sensitisation on behalf of RIFM. Therefore the data are accepted as dependable in spite of lacking documentation.
Flag: Critical study for SIDS endpoint
15-JAN-2004 (34)

5.4 Repeated Dose Toxicity

Type: Sub-chronic
Species: rat **Sex:** male/female
Strain: other: HsdBrl:WH (Wistar Hannover)
Route of administration: gavage
Exposure period: 28 days
Frequency of treatment: once daily
Post exposure period: 14 days

Doses: 0 (vehicle controls), 50, 250 and 1000 mg/kg bw/d
Control Group: yes, concurrent vehicle
NOAEL: = 50 mg/kg bw

Method: OECD Guide-line 407 "Repeated Dose Oral Toxicity - Rodent: 28-day or 14-d Study"
Year: 1997
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: Test laboratory
The test was performed at Quintiles England Ltd, Ledbury (England).
Animals
44 male and 44 female HsdBrl:WH (Wistar Hannover) strain rats of 3 to 4 weeks of age were supplied to the test lab by Harlan UK Ltd, Bicester (England). All animals were found to be healthy on arrival. They were then acclimatised in the experimental room for 11 days before the start of test. At the end of this period they were re-examined and confirmed to be healthy.
Allocation to treatment groups
Eight days before start of the test, all animals were weighed and the required number was selected by excluding those at the extremes of the weight range. The remaining animals were then randomly assigned to four test groups using a stratified body weight procedure: 6 males and 6 females to 50 mg/kg bw/d; 6 m & 6 f to 250 mg/kg bw/d; 12 m & 12 f to 1000 mg/kg bw/d; 12 m & 12 f to 0 mg/kg bw/d (maize/corn oil only, vehicle controls). After allocation, each animal was uniquely identified by subcutaneous implant of a transponder. Treatment groups were further identifed by colour markers on their respective cages. The last 6 animals of each sex in groups 1000 and 0 (controls) mg/kg bw/d were tagged to be maintained for an additional 14-day treatment-free period in order to follow reversibility of effects.
Environment and housing
The experimental room (designated E7) was air-conditioned and recorded temperatures were within the specified range of 19-25 °C; relative humidity was between 37% and 55%, within the specified range of 50±20 %RH; fluorescent lighting was automatically controlled to give a range of 12 hours light (from 06:00 to 18:00) and 12 hours dark.
The animals were housed in groups of 6 each in treatment-group-labelled, grid-bottomed stainless-steel cages of approximately 2000 cm2 surface, supended over paper-lined trays.
All animals had free access to pelleted SQC Rat and Mouse Maintenance Diet No. 1, Expanded (Special Diet Services, Witham, England) and mains tap water from polypropylene bottles. Each batch of diet was accompanied by a full certificate of analysis from the manufacturer, the mains water was regularly analysed for microbiological purity and levels of metals and halogenated hydrocarbons by independent analysts (Hyder Environmental, Bridgend, England). All certificates of analysis conformed to the respective specifications, are archived at the test facility and appended to the full test report.
Test article formulation
Pseudoionone was formulated as a solution in Maize (corn) Oil

B.P., with separate formulations prepared daily for each dose level. To confirm the achieved concentrations, samples of each formulation, including that of the vehicle control group, prepared on day 1 of weeks 1 and 4 of dosing were sent to the sponsor for analysis. Results of these analyses are included in the full test report.

Dosing

A constant volume of 5 ml/kg bw/d was used for all four groups. Individual doses were adjusted according to the most recent body weight recorded.

Observations

All animals were examined twice daily for mortality and morbidity. All visible signs of reactions to treatment were recorded daily. All animals were weighed at the start of the study and then twice weekly up to and including the day of killing and necropsy.

Clinical laboratory studies

Blood and urine samples were obtained from the first 6 males and females in each group during week 4 of treatment. Further blood and urine samples were obtained from the remaining animals towards the end of week 2 of the treatment-free period. Haematological examinations included diverse morphological, volume, coagulation and blood chemistry parameters, similarly for urinalyis; details are given in the full report.

Killing and terminal observations

At the end of the treatment and treatment-free periods, the designated animals were killed by carbon dioxide asphyxiation. All necropsies were completed within 2 days at the end of the treatment period and in 1 day after the treatment-free period. Each animal was weighed and examined externally. The abdominal cavity was opened and the animals were exsanguinated from the caudal vena cava. A macroscopic examination was then performed of the appearance of the organs in situ, from the cranial, thoracic and abdominal cavities. Any abnormalities were recorded. After trimming of fat and surrounding connective tissue, the following organs were weighed: adrenals, brain, epididymides, heart, kidneys, liver, ovaries, spleen, testes and thymus. Samples for histology were taken from 39 different organs or parts thereof (full list in report) as well as from all gross lesions. Subsequently, all tissues from all control and 1000-mg/kg-bw/d animals, including those allocated to the treatment-free period, plus all gross lesion samples from all animals, were wax-embedded, cut at 5 µm, stained with haematoxylin and eosin and examined microscopically.

Statistics

The study was designed for four groups per sex. The observations analysed were bodyweight before dosing (week 1), bodyweight gains over the interval week 1 to week 5 (regular dosing duration) and week 5 to week 7 (treatment-free period), food consumption over the same intervals and absolute as well as body-weight-related organ weights. Clinical pathology data were also analysed. The sexes were analysed separately. The data were subjected to analysis of variance, with further tests to assess potential group differences (Levene's test) and pairwise comparison of all treatment groups with controls (Williams's test). Statistical significance was accepted at 5% for two-sided tests and also noted at 1% and 0.1%. If the comparison of the high dose with controls was not significant, further statistical testing was stopped, otherwise the process

continued with one-sided tests.
The following results were analysed non-parametrically: After week 4 haematology (basophils, eosinophils, monocytes and white blood corpuscles in males; eosinophils and monocytes in females); biochemistry (albumin/globulin ratio, bilirubin, creatinine, gamma-glutamyl transpeptidase and potassium in males; albumin/globulin ratio and bilirubin in females); urinalysis (specific gravity in males). After week 6 biochemistry (gamma-glutamyl transpeptidase in males and females), but no haematological nor urinalysis data.

Result: Mortalities
There were no mortalities in this study.
Clinical observations
Salivation was recorded on a number of occasions pre-dosing in some animals of the 250 and 1000 groups and post-dosing in all animals of the 250 and 1000 groups. These findings were considered to be treatment-related. Several other clinical signs were recorded, none of which was considered to be related to treatment.
Bodyweight
Males (but not females) of the 1000 group showed a marked, significant reduction in bodyweight gain at the end of the treatment period. During the treatment-free period (-25%), bodyweight gains of males from the 1000 and 0 groups were comparable, however. There was no apparent effect on the bodyweights of females during treatment, however, over the treatment-free period the females from the 1000 group gained less weight than their comparable controls; this finding was considered fortuitous.

Dose group, mg/kg bw/d		Group mean bodyweight gain (g, %), day 1-29, statistically analysed			
		males		females	
		g	%	g	%
0	mean	114.4	100.0	58.3	100.0
	SD	13.7		10.3	
	n	9		9	
50	mean	110.7	96.8	50.3	86.3
	SD	14.2		32.4	
	n	3		3	
250	mean	107.0	93.5	60.7	104.1
	SD	6.2		7.4	
	n	3		3	
1000	mean	85.8***	75.0	55.9	95.9
	SD	16.0		11.8	
	n	9		9	

***: p < 0.001.

Food consumption
There were no treatment-related effects on food consumption.
Haematology
There was a slight increase in the group mean red blood cell (+6.6%) and packed cell volume values (+7.2%) for females of

the 1000 group. Increased group mean platelet values were also noted in males of the 1000 group, compared to the control group mean. However, on review of the data this was considered to reflect the low value for one control animal rather than any response to treatment. Regarding those coagulation parameters measured, there was a small but statistically significant increase in activated partial thromboplastin time in males of the 250 and 1000 groups, while no clear tend was visible in females:

Dose group mg/kg bw/d	Activated partial thromboplastin time, % of controls	
	males	females
0	100	100
50	96.7	85.0
250	124.7	109.0
1000	128.0	92.8

At the end of the treatment-free period, a light but statistically significant increase in packed cell volume was still observable in females of the 1000 group:

Dose group mg/kg bw/d	Relative red blood cell count (RBC) and packed cell volume (PCV), % of controls			
	males		females	
	relRBC	PCV	relRBC	PCV
0	100	100	100	100
50	102.5	101.6	98.7	100.2
250	101.3	100.0	101.3	103.6
1000	103.8	102.9	106.6	107.2

Although the mean activated partial thromboplastin value for males of the 1000 group and the mean red blood cell value for females of the 1000 group were still higher than their respective control groups, the differences were slight, did not achieve statistical significance and these parameters were considered to have recovered.

Blood chemistry

Small, statistically significant increases in alanine aminotransferase were observed in males from the 250 and 1000 groups (+36.7% and +53.3%, respectively) and in females from the 1000 group (+65%). An increase in gamma-glutamyl transpeptidase in both sexes was observed in the 1000 group (3 U/l compared to the control value of 0 U/l). Slight increases in total protein (+9.2%), globulin (+14.3%) and cholesterol levels (+36.8%) were observed in females from the 1000 group. Triglycerides were reduced in males of the 1000 group (-57.3%). Other, minor changes were observed which were within quoted background ranges and therefore not considered related to treatment. At the end of the treatment-free period in the 1000 group no findings were recorded that were considered of toxicological significance.

Urinalysis

After 4 weeks of treatment, minor changes in urobilinogen,

volume and protein levels were observed but these were considered to be coincidental and not related to treatment.
Organ weights
Absolute and bodyweight-related liver and kidney weights were increased in males (bw-related: +37.5% and +35.9%, respectively) and females (bw-related: +50.9% and +8.5%, respectively) of the 1000 group. Increased relative kidney weights were also seen in males of the 250 group (+14.5%).

Dose group, mg/kg bw/d	Bodyweight-related organ weights (%bw), statisically analysed group mean values at the end of week 4							
	liver				kidney			
	males		females		males		females	
	%bw	%rel	%bw	%rel	%bw	%rel	%bw	%rel
0 mean	4.80	100.0	4.36	100.0	0.675	100.0	0.847	100.0
SD	0.19		0.20		0.043		0.057	
n	6		6		6		6	
50 mean	4.96	103.3	4.16	95.9	0.668	99.0	0.778	91.9
SD	0.22		0.50		0.049		0.029	
n	6		6		6		6	
250 mean	5.06	105.4	4.74	109.2	0.730*	114.5	0.797	94.1
SD	0.18		0.36		0.035		0.077	
n	6		6		6		6	
1000 mean	6.60+	137.5	6.55+	150.9	0.917+	135.9	0.919*	108.5
SD	0.54		0.35		0.066		0.030	
n	6		6		6		6	

*: p < 0.05;
+: p < 0.001.

A number of other, statistically significant relative organ weight changes were observed in males of the 1000 group, however, these were considered to be due to the reduced overall bodyweight and not directly related to treatment with the test article. At the end of the treatment-free period there were no significant differences from controls for liver or kidney weight in both sexes. There were no other changes considered to be of toxicological significance.
Necropsy
No treatment-related abnormalities were observed.
Histopathology
No treatment-related abnormalities were observed. A small number of histological findings were within the normal range of background alterations seen in untreated rats of this age and strain.

Test substance: Pseudoionone from Teranol AG, Lalden, lot no. 14106, analysis no. 892E6, 15-Oct-1996:

Pseudoionone (cis + trans)	91.0%
Pseudoionone isomers 1+2	2.1%
6-Methylhept-5-en-2-one	0.3%
3,7-Dimethyloct-6-en-1-yn-3-ol	1.3%
C16-components	2.1%

Sum of other impurities 3.2%

Conclusion: Daily oral administration by gavage of pseudoionone to HsdBrl:WH (Wistar Hannover) rats for 28 days at a dose of 1000 mg/kg bw/d was associated with the following findings: intermittent pre- and post-dose salivation, reduction in bodyweight gain in males, an increase in liver and kidney weights and a few minor changes in haematology and blood chemistry. Administration of 250 mg/kg bw/d was associated with post-dose salivation on a number of occasions and a slight increase in relative kidney weight in males. Administration of 50 mg/kg bw/d did not result in any toxicological findings. In the 1000-mg/kg-bw/d group, there were no residual observations at the end of the 2-week treatment-free period, which shows that even the effects noted at 1000 mg/kg bw/d were of a transitory nature.

Reliability: (1) valid without restriction
OECD test under GLP with detailed report. Reliability 1.

Flag: Critical study for SIDS endpoint

10-JAN-2006 (93)

Type: Sub-chronic
Species: rat **Sex:** male/female
Strain: other: Wistar Crl: (WI) BR (outbred, SPF quality)
Route of administration: gavage
Exposure period: males: mean 106 (range 104-108) days;
females: mean 60 (range 36-65) days
Frequency of treatment: once daily
Post exposure period: none
Doses: 0 (vehicle controls), 40, 120 and 360 mg/kg bw/d
Control Group: yes, concurrent vehicle
NOAEL: = 120 mg/kg bw
LOAEL: = 360 mg/kg bw
NOEL : = 40 mg/kg bw

Method: other: OECD 415, One-generation reproductive toxicity
Year: 2003
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: For detailed methods, please refer to 5.8.1, Toxicity to Fertility

Result: Mortalities
There were 3 unscheduled deaths out of a total of 192 main parental animals; all 3 animals were females. Two were killed in extremis, one each in the 120 and the 360 mg/kg bw/d groups after 38 respectively 43 days of treatment. The other animal, also a female from the 360 mg/kg bw/d group, died spontaneously on day 38. All three were found to have severe delivery difficulties, with 17 foetuses in the birth canal, 16 dead pups and three foetal resorptions, and 19 foetuses in the birth canal, respectively. These deaths were considered incidental and very possibly caused by the big litter sizes. Therefore, these deaths were considered not to be related to the treatment with the test substance.
Clinical signs
Salivation was observed in all males and females of the highest dose group. Incidental findings consisted of alopecia, lethargy, clonic spasms, rales, salivation, scabs, nodule at the tail, red staining of the right eye, broken teeth, hunched posture, piloerection, pale appearance, emaciation, dull eyes

and dark eyes. No relationship was established with treatment for these observations or they were considered to be within the normal biological variation for rats of this age and strain. Animal no. 40 of group 2 (40 mg/kg bw/d) showed several signs of stress (compulsive biting, saltator spasms, tremor and muscle twitching) just before or after dosing during four days of treatment.
Body weight
Body weights and body weight gain rates were unaffected by treatment up to 360 mg/kg bw/d.
Food consumption
Statistically significant increases in relative food consumption were observed in some of the 120 and 360 mg/kg bw/d males. No explanation for this increase can be given, however, this finding was not considered an adverse effect, it was considered incidental in nature and not to be toxicologically relevant.
Macroscopic examination
No treatment-related macroscopic findings were identified but a number of findings that were considered incidental in nature. These findings included pelvic dilation of the left, right or both kidneys, testes reduced in size, flaccid testes, enlarged testes, accentuated lobular pattern of the liver, pale discolouration of the liver, alopecia at several parts of the body, dark red discolouration of the mediastinal cranial lymph nodes, isolated yellowish hard nodule at the tail of the left epididymis, dark red hard nodule at the left and right tips of the epididymides, epididymides reduced in size, enlarged liver, reddish soft nodule at the papillary process of the liver, soft nodule at the papillary process of the liver, stomach and spleen grown together with a soft nodule at the papillary process of the liver, dark red discolouration of the left mandibular lymph node. These findings are occasionally seen among rats used in this type of study and, in the absence of correlated microscopic histopathological findings, were not considered of toxicological significance.
Fluid in the uterus (in one female of the control group, in three females of the 40 mg/kg bw/d group, in one female of the 120 group and in one female of the 360 group) is related to a stage in the oestrous cycle and is a normal finding.
In the 120 mg/kg bw/d group, one female that was killed in extremis showed 17 foetuses in the birth canal. Of the 360 group, one female that was killed in extremis showed 3 foetal resorptions and 9 placentas in the left uterus horn and the thoracic cavity containing milky-cloudy fluid; one female from the 360 group that died spontaneously showed 19 foetuses in the birth canal and beginning autolysis.
Organ weights
Males and females of the 360 mg/kg bw/d group showed statistically significant increased absolute and relative liver and kidneys weight. Males of the 120 group showed significantly increased liver weight. In the absence of histopathological changes, both effects were considered not to be toxicologically relevant but rather manifestations of physiological adaptation to additional metabolic and excretionary loads. Males of the 40 group showed statistically significantly reduced seminal vesicles weight. In the absence of a dose-response relationship, this finding was considered to be caused by chance and not to be related to treatment.
Microscopic examination

There were no treatment-related findings. No histopathological changes were found to correlate with the observed increase in liver and kidney weights.

Test substance: Pseudoionone from Teranol AG, Lalden, batch no. UU02033826, purity 95.4% (area, GC).

Conclusion: No effects that were regarded as adverse were seen at 120 mg/kg bw/d during 106 days in males respectively 60 days in females. Based on this test, which was of subchronic duration for the females and of chronic duration for the males, the NOAEL was 120 mg/kg bw/d and the NOEL was 40 mg/kg bw/d.

Reliability: (2) valid with restrictions
While this was not a subchronic or chronic toxicity study but a reprotoxicity test, it was performed according to a stringent protocol under GLP, animals were observed daily and dissected after killing. Full single data are reported. Reliability 2.

Flag: Critical study for SIDS endpoint

19-NOV-2003 (9)

Type: Sub-chronic
Species: rat **Sex:** no data
Strain: other: former Roche inbred strain
Route of administration: gavage
Exposure period: 5 days
Frequency of treatment: once daily
Post exposure period: 10 days
Doses: 500, 1000, 2000, 4000 and 8000 mg/kg bw/d
Control Group: yes, historical

Method: other: former Roche gavage oral toxicity test
Year: 1975
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: As usual for this internal Roche testing scheme, groups of 10 animals per dosage were used. Administration was by gavage, once daily for five consecutive days. Observation was 10 days after administration, then the test animals were killed and dissected. Statistics were computed if applicable. Controls were historical with the same rat strains.

Result:

Pseudoionone, daily dose, mg/kg bw	Cumulative deaths, %, on day 1	day 2	day 3	day 4	day 5	day 15
8000	0	0	70	100	100	100
4000	0	0	10	20	50	60
2000	0	0	0	0	0	0
1000	0	0	0	0	0	0
500	0	0	0	0	0	0

Based on the deaths noted, the following oral lethal dose values in mg/kg bw were interpolated:

	24 h post first dose	24 h and 10 d post 5th dose
LD10	>8000	2540 2470
LD50	>8000	4000±640 3880±620
LD90	>8000	6280 6080

A slight reduction in body weight gain of the survivors between day 1 before the first administration and day 6, 24 hours after the fifth administration, was seen with the 2000 and 4000 mg/kg bw/d doses. Symptoms are described as sedation for the doses 8000, 4000 and 2000 mg/kg bw/d and light sedation for 1000 and 500 mg/kg bw/d.

Test substance: Pseudoionone from Teranol, Lalden, Mag-No 4 3591 0.
Reliability: (2) valid with restrictions
While this test is reported only in very abbreviated form, the acute toxicity group led by the author of the report performed large series of highly standardised toxicity tests in the late 1960s, 1970s and early 1980s. Serial testing in a dedicated facility assures dependably regular animal keeping, test substance administration, laboratory protocol and reporting. Therefore these internal data are regarded as valid and dependable.
11-FEB-2003 (14)

Type: Sub-chronic
Species: mouse **Sex:** no data
Strain: other: former Roche inbred strain
Route of administration: gavage
Exposure period: 5 days
Frequency of treatment: once daily
Post exposure period: 10 days
Doses: 1000, 2000, 4000 and 8000 mg/kg bw/d
Control Group: yes, historical

Method: other: former Roche gavage oral toxicity test
Year: 1973
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: As usual for this internal Roche testing scheme, groups of 5 or 10 animals per dosage were used. Administration was by gavage, once daily for five consecutive days. Observation was 10 days after administration, then the test animals were killed and dissected. Statistics were computed if applicable. Controls were historical with the same mouse strains.
Result: Pseudoionone, Cumulative deaths, %, on

daily dose, mg/kg bw	day 1	day 2	day 3	day 4	day 5	day 15
8000	80	100	100	100	100	100
4000	0	0	0	0	10	10
2000	0	0	0	0	0	0
1000	0	0	0	0	0	0

Based on the deaths noted, the following oral lethal dose values in mg/kg bw were interpolated:

	24 h post single dose	24 h and 10 d post 5th dose
LD10	4750	3060 3060
LD50	7270±1100	4550±640 4550±640
LD90	>8000	6770 6770

A slight reduction in body weight gain of the survivors between day 1 before the first administration and day 6, 24 hours after the firth administration, was seen with higher doses.
Reliability: (2) valid with restrictions
While this test is reported only in very abbreviated form, the acute toxicity group led by the author of the report performed large series of highly standardised toxicity tests in the late 1960s, 1970s and early 1980s. Serial testing in a dedicated facility assures dependably regular animal keeping, test substance administration, laboratory protocol and reporting. Therefore these internal data are regarded as valid and dependable.
11-FEB-2003 (13)

5.5 Genetic Toxicity 'in Vitro'

Type: Ames test
System of testing: TA1535, TA97, TA98, TA100, TA102, with and without S9 metabolic activation
Concentration: 0 (control), 1.6, 5, 16.6, 50, 166 and 500 µg/plate
Cytotoxic Concentration: 500 µg/plate
Metabolic activation: with and without
Result: negative

Method: OECD Guide-line 471
Year: 1996
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: Media
All media were prepared according to the OECD guideline. Full details are in the test report. Pseudoionone was dissolved in DMSO and then serial dilutions with DMSO were prepared to add to bacterial media.
Metabolic activation system
Male albino randomised-strain SPF rats from BRL Laboratories (Füllinsdorf, Switzerland) were treated by i.p. injections with phenobarbital/beta-naphthoflavone (details and doses in report) for 4 days and killed on the fifth. The livers were removed under aseptic ocnditions and liver homogenates prepared according to the guideline. S9 fractions were prepared from the pooled homogenates by centrifugation, collected in 2-ml cryotubes and deep-frozen (-70±10 °C) until use. S9 mixture was freshly prepared from the S9 fractions with specified solvents and buffers.
Bacterial strains
Salmonella typhimurium strains TA1535, TA97, TA98, TA100 and TA102 were originally obtained from BN Ames. They were stored in nutrient broth (NB) cultures supplemented with 9% dimethylsulfoxide (DMSO) in liquid nitrogen, according to Ames et al. (Methods for detecting carcinogens and mutagens with Salmonella/mammalian microsome mutagenicity test. Mutat Res 31: 347-364, 1975). For use in tests, cultures of the strains were grown in NB medium at 37 °C overnight in a shaking water bath. The sensitivity of the strains was verified using positive control substances (sodium azide, ICR191, 2-nitrofluorene, MMC), moreover, all strains were grown in the presence of 2-aminoanthracene with and without S9 mix in order to check the activity of the latter.
Test procdure
1) Toxicity prescreen
A toxicity prescreen was performed with strain TA100 in duplicate doses and solvent only controls. Toxic effects, as measured by reduced background growth and reduction in the number of revertant colonies, were observed starting at 500 µg/plate. This was chosen as the highest test concentration.
2) Standard Ames procedure
Test tubes containing 2 ml agar medium were autoclaved and then histidine/biotin mixture, 0.1 ml of pseudionone diluted with DMSO or solvent only or 0.05 ml of reference substances (see above), 0.1 ml of the overnight culture broth for the respective strain and 0.5 ml of the S9 mix where scheduled respectively 0.5 ml sodium-buffered saline pH 7.4 were mixed and immediately poured onto Vogel-Bronner minimal agar plates.

Targeted pseudoionone concentrations were 50, 166 and 500 µg/plate. Three replicate plates for every test compound concentration and the negative control plus two replicate plates for every positive control were incubated upside down at 37 °C for 2 days.
3) Liquid pre-incubation assay
0.1 ml of pseudoionone diluted with DMSO or solvent only or 0.05 ml of reference substances (see above), 0.5 ml of the S9 mix where scheduled respectively 0.5 ml sodium-buffered saline pH 7.4 and 0.1 ml of the overnight culture broth for the respective strain were mixed and incubated on a shaker for 30 minutes at 37 °C. Then soft agar supplemented with histidine/biotin was added, the tubes mixed and the contents immediately poured onto Vogel-Bronner minimal agar plates. Further procedure as above.
Data reporting
Colonies were counted electronically using a Domino image analysis system (Perceptive Instrument, Halstead, England). The background lawn was inspected using a microscope for toxicity; absence or presence of a confluent bacterial lawn was recorded and interpreted as toxicity or non-toxicity fo the test substance.

Result: Strain-dependent toxicity was noted with both methods used. In the liquid preincubation assay, toxicity was noted already at 50 µg/plate for some strains in the absence of S9 mix. Therefore a repeat experiment using the preincubation method was performed in the concentration range of 0.5-50 µg/plate with strain TA1535 and TA102, with and without S9 mix. Neither in the standard Ames plate incorporation assay nor in the liquid preincubation assay were increases in mutant colony frequency noted with any of the 5 strains, with or without S9 mix. The mutant frequencies of the controls were in the historical range of controls.

Test substance: Pseudoionone from Teranol AG, Lalden, Lot no. 05076, Analysis no. 575E6, purity 91.2% (area, GC).

Conclusion: Neither pseudoionone per se nor any of its S9-mix metabolites were mutagenic in standard Ames and liquid preincubation bacterial mutagenicity assays with five different strains of Salmonella typhimurium.

Reliability: (1) valid without restriction

Flag: Critical study for SIDS endpoint

12-FEB-2003 (1)

Type: Ames test
System of testing: TA98, TA100, TA1535, TA1537, with and without S9 metabolic activation
Concentration: 3 µmol/plate (= 577 µg/plate) and blank/solvent controls
Cytotoxic Concentration: no data
Metabolic activation: with and without
Result: negative

Method: other: according to Ames et al. (1975): Mutat Res 31: 347 ff.
Year: 1980
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Test system
Compounds were tested in an Ames assay according to Ames et al (Mutat Res 31: 347 ff, 1975) with histidine-requiring

Salmonella typhimurium strains TA98, TA100, TA 1535 and TA1537, obtained directly from Dr BN Ames (University of California, Berkeley, CA, USA).
Initially, cultures were grown in Difco nutrient broth, which was later substituted by Oxoid nutrient broth because of concern about weak mutagenic activity expressed by BN Ames in a peronal communication to the authors. 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.
S9 fractions for metabolic activation were prepared as described by Ames et al. (see above). Aroclor 1254 or 3-methylcholanthrene, both suspended in maize/corn oil, were used as inducers in male Sprague-Dawley rats. Full details as to the preparation of the S9 mix are in the publication.
Test substances
Most compounds, including pseudoionone, were dissolved in ethanol for incorporation into the plates.

Result: Pseudoionone 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 (= 577 µg/plate).

Test substance: All test substances including pseudoionone were checked for purity using TLC, GC and NMR. Compounds containing more the 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
Detailed methods and quality as well as positive controls. As pseudoionone was not mutagenic with or without metabolic activation, this negative result is only stated summarily, which, however, is regarded as a valid procedure. Reliability 2.

12-FEB-2003 (33)

5.6 Genetic Toxicity 'in Vivo'

Type: Micronucleus assay
Species: mouse **Sex:** male
Strain: other: NMRI BR
Route of admin.: gavage
Exposure period: 24 and 48 hours
Doses: 2000 mg/kg
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: Animals
NMRI BR (SPF) mice from Charles River, Sulzfeld, Germany were used. Animals were young adults (6-8 weeks old), females were nulliparous and non-pregnant.
The animals were housed in an air-conditioned room with approximately 15 air changes per hour, a temperature of 21±3 °C and a relative humidity between 30 and over 70%; inspite of the relative humidity exceeding 70% for part of the test period, no abnormalities were noted in the animals and it was concluded that this deviation did not affect the integrity of the study. The animal room was illuminated for 12 hours per day with artificial fluorescent lighting and was dark for 12 hours.
The animals were housed in randomised groups of 5 each per sex per cage in labelled polycarbonate cages containing purified sawdust (Sawi, Jelu-Werk, Rosenberg, Germany) as bedding material. Paper bedding (BMI Helmond, The Netherlands) was provided for nest material. There was free access to standard pelleted diet (Altromin (code VRF 1), Lage, Germany) and to tap water. Certificates of analysis for all substrates, feed and water are retained in the NOTOX archives. For all animals there was an acclimatisation period of at least 5 days before start of treatment under laboratory conditions.
Treatment groups
3 males and 3 females were used for the dose range-finding test. 5 males each per test group respectively as negative and positive controls were used as there were no obvious differences between sexes in the range-finding test. All animals were identifed by a unique number on the tail.
In the main test there were 4 groups, labelled A through D. A was a negative control (vehicle only, 10 ml maize/corn oil/kg bw) group, B and C were treatment groups (2000 mg pseudoionone/kg bw in maize/corn oil, dose adjusted to a volume of 10 ml/kg bw; group B to be sampled at 24 hours post-dosing, group C at 48 hours post-dosing) and D was a positive control group (50 mg cyclophosphamide/kg bw, dissolved in physiological saline; cyclophosphamide from Asta-Werke, Germany). Feed was withheld 3-4 hours prior to dosing. Administration was by oral gastric intubation.
Observations
The animals were observed at least once a day for signs of toxicity. Prior to dosing the animals were weighed.
Preparation of erythroblasts and erythrocytes
The test animals were killed by cervical dislocation 24 hours (groups A and B) respectively 48 hours (groups C and D) after dosing. In every instance, both femurs were removed and freed of blood and muscles. Then, both ends of the bone were shortened until a small opening to the marrow canal became visible. The prepared bones were flushed with foetal calf serum (FCS), the cell suspension was collected and centrifuged at 1000 rpm for 5 minutes. The supernatant was discarded and the pellets re-suspended in FCS. A drop of the suspension was placed on the end of a previously cleaned and marked (NOTOX study number, animal number) microscopic slide, spread using a clean slide and air-dried, fixed with 100% methanl and automatically stained in a HEMA-tek Slide Stainer (Miles, Bayer Nederland, The Netherlands) and covered with a

glass coverslip.
Before analysis, the unique marks of each slide were randomised by covering with an adhesive label bearing the NOTOX study number and a code. Slides were first screened at a magnification of x100 for suitable regions, then scored at x1000. The number of micronucleated polychromatic erythrocytes was counted in a total of 2000 polychromatic erythrocytes per slide. The ratio of polychromatic to normochromatic erythrocytes was determined in the first 1000 erythrocytes scanned. Micronuclei were only counted in polychromatic erythrocytes.
Statistics
After counting, the randomisation was unveiled and averages and standard deviations for the four groups were calculated. A test substance and/or dose would be considered positive if it induced a statistically significant (Wilcoxon Rank Sum test, two-sided test at $P < 0.05$) increase in the frequency of micronucleated polychromatic erythrocytes, at any dose or sampling time. Conversely, a test substance is considered negative if there is no such statistically signifcant difference at any dose or sampling time.
Acceptability criteria
A micronucleus test is considered acceptable if it meets the following criteria: 1) the positive control substance, cyclophosphamide, induces a significant increase in micronucleated polychromatic erythrocytes and the incidence of micronucleated polychromatic erythrocytes in the control animals is reasonably within the laboratory historical controls range (mean ± 3 SD).

Result: Dose range-finding study
3 males and 3 females were dosed with 2000 mg pseudoionone in maize/corn oil per kg bw. All treated animals showed no abnormalities during an observation period of 3 days. Therefore, 2000 mg/kg bw was chosen as the only dose for testing. Moreover, as there were no obvious differences between the sexes, it was decided to use only males in the main test.
Micronucleus test
The mean bodyweights of all four groups, recorded just before dosing, were not statistically different (data available).
All animals treated with 2000 mg/kg bw showed no abnormalities; this was also true for both the negative and positive controls.
Average numbers of micronucleated polychromatic erythrocytes per 2000 polychromatic erythrocytes and ratios of polychromatic to normochromatic erythrocytes:

Group	Dose, mg/kg bw	Sampling time, h	Number, mean±SD	Ratio, mean±SD
A, vehicle control	0	24	2.2±1.5	1.16±0.13
B, Pseudoionone	2000	24	1.4±1.1	1.20±0.10
C, Pseudoionone	2000	48	1.8±1.5	1.07±0.06
D, Cyclophosphamide	50	24	44.4±10.6**	0.29±0.07

** Significantly different from negative (vehicle) control group, $P <= 0.01$.
All single data are available in the report.

Test substance: Pseudoionone from Teranol AG, Lalden, Switzerland, lot no. UU02033826, purity 95.4% area, GC), complying with specification. Certificate of analysis no. 554, dated 28-MAR-2002, Quality Control Department, Teranol, Lalden.

Conclusion: Pseudoionone at an oral dose of 2000 mg/kg bw did not induce any increase in the incidence of micronucleated polychromatic erythrocytes in this in vivo mouse test. Therefore, pseudoionone is regarded as negative regarding genotoxic effects in this model.
Further, the test groups treated with pseudoionone did not show any decrease in the ratio of normochromatic to polychromatic erythrocytes, which reflects a lack of toxic effects of pseudoionone on erythropoiesis.
Last, no animal in either the dose range finding study (3 males, 3 females) nor in the treatment groups (5 males 24 h; 5 males 48 h) died after a single oral dose of 2000 mg pseudoionone/kg bw.
Reliability: (1) valid without restriction
OECD study under GLP, reliability 1.
Flag: Critical study for SIDS endpoint
24-SEP-2004 (10)

5.7 Carcinogenicity

5.8.1 Toxicity to Fertility

Type: One generation study
Species: rat
Sex: male/female
Strain: other: Wistar Crl: (WI) BR (outbred, SPF quality)
Route of administration: gavage
Exposure Period: males: mean 106 (range 104-108) days;
females: mean 60 (range 36-65) days
Frequency of treatment: once daily
Premating Exposure Period
male: 11 weeks
female: 2 weeks
Duration of test: 126 days
No. of generation studies: 1
Doses: 0 (vehicle controls), 40, 120 and 360 mg/kg bw/d
Control Group: yes, concurrent vehicle
NOAEL Parental: = 120 mg/kg bw
NOAEL F1 Offspring: = 360 mg/kg bw

Method: OECD Guide-line 415 "One-generation Reproduction Toxicity Study"
Year: 2002
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: Animals
Male and female Wistar rats Crl: (WI) BR (outbred, SPF quality) were acquired from Charles River Deutschland, Sulzfeld, Germany. Of the animals assigned to the four groups, the 96 males were 5-6 weeks old and the 96 females were 11-12 weeks old. All animals were given a health check to ensure good state of health at the beginning of the study. All animals were acclimatised for at least 5 days before assignment by computer-generated randomisation according to body weight, with all animals within ±20% of the sex mean to treatment groups and start of the study. All animals were uniquely identified by tattoo on the tail.

Animal husbandry
All animals were housed in suspended stainless-steel cages in climate-controlled rooms at 21±3 °C, a relative humidity of 30-70% and a 12-hour-light/12-hour-dark cycle. Animals had free access to standard pelleted rat diet (Altromin, code VRF1, Lage, Germany) and tap water. Analyses for all batches of feed and quarter-yearly analyses of tap water are retained at NOTOX archives. On arrival, all animals were housed in groups of 4 animals per sex per cage, with males and females being kept in separate rooms. During mating, parental females were caged with parental males on a 1-to-1 basis in suspended stainless steel cages with wire mesh floors. Mated females and males were housed individually in labelled polycarbonate cages containing sawdust (SAWI bedding, Jelu-Werk, Rosenberg, Germany) as bedding material. During the final stage of the pregnancy period, from day 16 post coitum, and during lactation, paper (Enviro-dri, BMI, Helmond, The Netherlands) was supplied to the dams for incorporation into the nest. The paper was replaced when soiled.
Treatment
Pseudoionone was formulated daily using maize/corn oil as the vehicle. Formulations were analytically confirmed to be stable for at least 4 hours at room temperature and to correspond to targeted concentrations. Dosing was by oral gavage using a stainlees steel stomach tube, dose volume was 5 ml/kg bw, actual volumes were calculated according to the latest individual body weights. Dose levels were 0 (vehicle controls), 40, 120 and 360 mg/kg bw/d for the four groups; these dose levels were based on a GLP 28-day subchronic toxicity study with the same dose levels that resulted in a NOEL of 50 mg/kg bw/d and a LOAEL of 250 mg/kg bw/d with reversible effects. The males were exposed for 11 weeks prior to mating up to termination; the mean exposure was 106 days, with a range from 104 to 108 days. The females were exposed for 2 weeks prior to mating up to termination; the mean duration of treatment was 60 days, with a range of 36 to 65 days. The offspring was not treated.
Mating procedures
Main pairing. Females were paired on a one-to-one basis with males from the same treatment group. Each morning the trays under the mating cages were inspected for ejected copulation plugs. The day on which a copulation plug was found was designated as day 0 of gestation. Once mating had occurred, the males and females were separated. In case no copulation plug was detected within 3 weeks of pairing, the male and female were separated.
Parturition
The pregnant females were allowed to litter normally. Day 1 of lactation was defined as the day when a litter was found completed (ie, membranes, placentas cleaned up, nest built up and/or feeding of pups started). Females that were in the process of littering were left undisturbed.
Culling offspring
On day 4 after birth the size of each litter was adjusted at random by eliminating extra pups to yield, as closely as possible, four male and four female pups per litter. Elimination of runts only was not appropriate. Whenever the number of pups per sex did not allow four plus four, partial adjustement was made to come as close as possible to that ratio, eg, three males plus five females. No adjustment was

made for litters of eight pups or less.
Identification of offspring
Pups were identified individually by means of intracutaneous injection of Indian ink or by tattoo on the feet.
Termination
All survivors were killed by exsanguination after iso-flurane anaesthesia. The males were killed after confirmation of the pregancy of the female they had been mated with or after successful delivery of the respective dam. The females were killed at day 21 post partum or shortly thereafter. Pups were killed either at adjusting litters on day 4 post partum or at the end of the study at day 21 post partum.
Observations
Parental animals were observed twice daily for behavioural and clinical signs, the latter were recorded according to fixed scales. Cage debris of pregnant females were examined to detect abortion or premature birth. Signs of difficult or prolonged parturition were recorded. Males and females were weighed on the first day of exposure and weekly thereafter. Mated females were weighed on days 0, 7, 14 and 21 of gestation and during lactation on days 1, 4, 7, 14 and 21. Food consumption was recorded weekly for males and females, with exception of the mating period. Food consumption of mated females was recorded on gestation days 0, 7, 14 and 21 and during lactation on days 1, 4, 7, 14 and 21. Regarding water consumption, subjective appraisal was maintained during the study as there were no suspicions of any effect of treatment. Reproductive basic data such as numbers of animals mated, mating date, confirmation of pregnancy and day of delivery were recorded.
For the offspring, the numbers of live and dead pups at first litter check (= day 1 of lactation) and daily thereafter was recorded as well as the individual weight of all live pups on days 1, 4, 7, 14 and 21 of lactation, the sex of the pups by assessment of the ano-genital distance, the number of pups with physical or behavioural abnormalities.
Pathology
After killing or natural death all parental main animals were subjected to external examination and to macroscopic examination during dissection, specifically the cranial, thoracic and abdominal organs and tissues, with special attention to the reproductive organs. All macroscopic abnormalities were recorded. The additional animals were not subjected to macroscopic examination.
The terminal body weight and the following organ weights were recorded from the main parental animals on the day of death: cervix plus uterus, epididymides (both together), kidney, liver, ovaries, pituitary (weighed after 24 h fixation), prostate (weighed after 24 h fixation), seminal vesicles together with coagulating gland and fluids, spleen and testes.
During dissection, samples of the following organs and tissues were collected from all main parental animals and fixed in neutral, phosphate-buffered 4% formaldehyde solution: all gross lesions, cervix, coagulation gland, epididymides (fixed in Bouin's, transferred to formalin after 24 h), kidneys, liver, ovaries, pituitary, prostate, seminal vesicles, spleen, testes (fixed in Bouin's, transferred to formalin after 24 h), uterus and vagina. In case a female was not pregnant, the whole uterus was stained after Salewski in order to determine any early post-implantation losses through evidencing

implantation site scars.
Histopathology. All organ and tissue samples as listed below were processed, embedded, microtomed at 2-4 µm and stained with haematoxylin and eosin: kidneys and liver from 10 randomly selected animals per sex from all treatment groups. All slides were examined by a professional histopathologist, abnormalities were described and included in the histopathology report. The histopathologist was asked to add an interpretation of the findings.
Pups. Main offspring found dead or killed before day 14 of lactation were sexed and externally examined if practically possible. The stomach was examined for the presence of milk. Main offspring found dead or killed on or after day 14 of lactation were sexed and subjected to external examination of the thoracic and abdominal tissues and organs; all abnormalities were recorded. If possible, defects or cause of death were evaluated.
For variables assumed to follow a normal distribution, the Dunnett test was applied; for other assumed distributions the Steel test was used. In those cases where variables could be dichotomised without loss of information, the exact Fisher test was applied. All tests were two-sided, significance was accepted at $p < 0.05$.

Result: Protocol deviations
13 protocol deviations are listed in the report. All 13 were evaluated and considered not to have affected the integrity of the study or of the results.
Dose preparations
A first analysis of formulations prepared on 17-Jun-2002 showed values for accuracy within the range of 86-131% for pseudoionone peak 1 and of 84-126% for peak 2, which was considered insufficient. Additional analyses were performed and the one of the formulations prepared on 24-Jun-2002 showed 98-102% for peak 1 and 97-102% for peak 2. The insufficient results were considered to originate from pipetting errors of the volatile solvent (n-hexane) during sample pretreatment for chemical analysis. Preparations of the formulations, however, were performed according to the accurate method and it was concluded that the animals received the complete and correct exposure to the test substance.
Analyses for homogeneity of the low- and high-dose formulations prepared on 17-Jun-2002, 24-Jul-2002 and 29-Aug-2002 all showed values within the range of 94-109% for peak 1 and of 84-115% for peak 2, which were considered acceptable for this type of formulations.
A stability analysis of the low- and high-dose formulations from 17-Jun-2002 showed decreases over 7 days of 15% (peak 1) and and 11% (peak 2) for groups 40 mg/kg bd/d and of 15% (peak 1) and 13% (peak 2) for group 360 mg/kg bw/d, which was considered sufficient in view of the fact that formulations were prepared daily.
Mortalities
There were 3 unscheduled deaths out of a total of 192 main parental animals; all 3 animals were females. Two were killed in extremis, one each in the 120 and the 360 mg/kg bw/d groups after 38 respectively 43 days of treatment. The other animal, also a female from the 360 mg/kg bw/d group, died spontaneously on day 38. All three were found to have severe delivery difficulties, with 17 foetuses in the birth canal, 16 dead pups and three foetal resorptions, and 19 foetuses in the

birth canal, respectively. These deaths were considered incidental and very possibly caused by the big litter sizes. Therefore, these deaths were considered not to be related to the treatment with the test substance.
Clinical signs
Salivation was observed in all males and females of the highest dose group. Incidental findings consisted of alopecia, lethargy, clonic spasms, rales, salivation, scabs, nodule at the tail, red staining of the right eye, broken teeth, hunched posture, piloerection, pale appearance, emaciation, dull eyes and dark eyes. No relationship was established with treatment for these observations or they were considered to be within the normal biological variation for rats of this age and strain. Animal no. 40 of group 2 (40 mg/kg bw/d) showed several signs of stress (compulsive biting, saltator spasms, tremor and muscle twitching) just before or after dosing during four days of treatment.
Body weight
Body weights and body weight gain rates were unaffected by treatment up to 360 mg/kg bw/d.
Food consumption
Statistically significant increases in relative food consumption were observed in some of the 120 and 360 mg/kg bw/d males. No explanation for this increase can be given, however, this finding was not considered an adverse effect, it was considered incidental in nature and not to be toxicologically relevant.
Macroscopic examination
No treatment-related macroscopic findings were identified but a number of findings that were considered incidental in nature. These findings included pelvic dilation of the left, right or both kidneys, testes reduced in size, flaccid testes, enlarged testes, accentuated lobular pattern of the liver, pale discolouration of the liver, alopecia at several parts of the body, dark red discolouration of the mediastinal cranial lymph nodes, isolated yellowish hard nodule at the tail of the left epididymis, dark red hard nodule at the left and right tips of the epididymides, epididymides reduced in size, enlarged liver, reddish soft nodule at the papillary process of the liver, soft nodule at the papillary process of the liver, stomach and spleen grown together with a soft nodule at the papillary process of the liver, dark red discolouration of the left mandibular lymph node. These findings are occasionally seen among rats used in this type of study and, in the absence of correlated microscopic histopathological findings, were not considered of toxicological significance.
Fluid in the uterus (in one female of the control group, in three females of the 40 mg/kg bw/d group, in one female of the 120 group and in one female of the 360 group) is related to a stage in the oestrous cycle and is a normal finding.
In the 120 mg/kg bw/d group, one female that was killed in extremis showed 17 foetuses in the birth canal. Of the 360 group, one female that was killed in extremis showed 3 foetal resorptions and 9 placentas in the left uterus horn and the thoracic cavity containing milky-cloudy fluid; one female from the 360 group that died spontaneously showed 19 foetuses in the birth canal and beginning autolysis.
Organ weights
Males and females of the 360 mg/kg bw/d group showed statistically significant increased absolute and relative

liver and kidneys weight. Males of the 120 group showed significantly increased liver weight:

Dose group, mg/kg bw/d		Bodyweight-related organ weights (%bw), group mean values			
		liver		kidney	
		males	females	males	females
0	mean	3.33	4.28	0.68	0.69
	SD	0.23	0.31	0.05	0.04
	n	24	24	24	24
40	mean	3.39	4.16	0.68	0.69
	SD	0.19	0.45	0.06	0.05
	n	24	22	24	22
120	mean	3.61**	4.45	0.71	0.70
	SD	0.20	0.25	0.07	0.05
	n	24	23	24	23
360	mean	4.13**	4.75**	0.77**	0.73*
	SD	0.29	0.29	0.07	0.06
	n	24	22	24	22

*/**: Dunnett's test on pooled variance, significant at 5% (*) or 1% (**) level.

In the absence of histopathological changes, both effects were considered not to be toxicologically relevant but rather manifestations of physiological adaptation to additional metabolic and excretionary loads.
Males of the 40 group showed statistically significantly reduced seminal vesicles weight. In the absence of a dose-response relationship, this finding was considered to be caused by chance and not to be related to treatment.
Microscopic examination
There were no treatment-related findings. No histopathological changes were found to correlate with the observed increase in liver and kidney weights.
Reproduction
Reproduction parameters were unaffected by treatment up to 360 mg/kg bw/d. In the 40 group, one female did not mate and one female was non-pregnant. In the 120 group, one female showed delivery difficulties, and in the 360 group, two females showed delivery difficulties. Mating performance, duration of gestation and fertility parameters including number of pups at birth were similar for the control and treated groups.

Test substance: Pseudoionone from Teranol AG, Lalden, batch no. UU02033826, purity 95.4% (area, GC).

Conclusion: Gavage treatment of male and female Wistar rats with pseudoionone at dose levels 0 (controls), 40, 120 and 360 mg/kg bw/d revealed parental and breeding (post partum F1) toxicity at 360 mg/kg bw/d. Reproductive parameters and development of the pups were unaffected up to 360 mg/kg bw/d. Based on the results of this one-generation study, the parental NOAEL was established at 120 mg/kg bw/d and the reproductive NOAEL was 360 mg/kg bw/d.

Reliability: (1) valid without restriction

Recent study according to OECD Guideline 415 under GLP, with full report and all individual data. Reliability 1.
Flag: Critical study for SIDS endpoint
10-JAN-2006 (9)

5.8.2 Developmental Toxicity/Teratogenicity

Species: rat **Sex:** male/female
Strain: other: Wistar Crl: (WI) BR (outbred, SPF quality)
Route of administration: gavage
Exposure period: males: mean 106 (range 104-108) days;
females: mean 60 (range 36-65) days
Frequency of treatment: once daily
Duration of test: 126 days
Doses: 0 (vehicle controls), 40, 120 and 360 mg/kg bw/d
Control Group: yes, concurrent vehicle
NOAEL Maternal Toxity: = 120 mg/kg bw
NOAEL Teratogenicity: = 360 mg/kg bw

Method: other: OECD Guideline 415, One-generation reproductive toxicity
Year: 2002
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: For detailed methods, please see 5.8.1, Toxicity to Fertility.
Result: For general results, please refer to 5.8.1, Toxicity to Fertility.
Reproduction
Reproduction parameters were unaffected by treatment up to 360 mg/kg bw/d. In the 40 group, one female did not mate and one female was non-pregnant. In the 120 group, one female showed delivery difficulties, and in the 360 group, two females showed delivery difficulties. Mating performance, duration of gestation, fertility parameters and number of pups at birth were similar for the control and treated groups.

Dose group, mg/kg bw/d		Litter size, n	live births, n	mean bodyweights, g
0	mean	14.7	14.5	6.8
	SD	2.5	2.72	0.62
	n	24	24	24
40	mean	15.0	14.1	6.6
	SD	2.5	3.98	0.44
	n	22	22	21
120	mean	15.7	15.1	6.5*
	SD	1.3	2.72	0.45
	n	23	23	23
360	mean	15.4	14.5	6.5
	SD	4.5	5.38	0.59
	n	23	23	22

*: $p < 0.05$, Dunnett's test on pooled variance.

Pups

Development of the pups was unaffected by treatment up to 360 mg/kg bw/d. Numbers of pups at birth were similar between controls and all treatment groups. No teratogenic malformations are reported. However, postnatal deaths were significantly increased at 360 mg/kg bw/d during days 0-4 post partum, due to which the viability index was decreased in this group (91.0 compared to 96.6 in controls).

Test substance: Pseudoionone from Teranol AG, Lalden, batch no. UU02033826, purity 95.4% (area, GC).

Conclusion: In a reproductive study by gavage treatment of male and female Wistar rats with pseudoionone at dose levels 0 (controls), 40, 120 and 360 mg/kg bw/d, reproductive parameters and development of the pups were unaffected up to 360 mg/kg bw/d. Specifically, no foetal malformations were recorded. Based on the results of this one-generation study, the reproductive and developmental NOAEL was 360 mg/kg bw/d.

Reliability: (1) valid without restriction
Recent study according to OECD Guideline 415 under GLP, with full report and all individual data. Reliability 1.

Flag: Critical study for SIDS endpoint

10-JAN-2006 (9)

Species: hamster **Sex:** female
Strain: other: LAK:LVG(SYR)
Route of administration: gavage
Frequency of treatment: once only, on day 8 of gestation
Duration of test: 6 days, from day 8 to day 14 of gestation
Doses: 96 and 960 mg/kg bw
Control Group: yes, concurrent vehicle
NOAEL Maternal Toxity: = 96 mg/kg bw
NOAEL Teratogenicity: = 960 mg/kg bw
Result: not teratogenic

Year: 1985
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Animals and husbandry
Female timed pregnant Syrian hamsters of strain LAK:LVG(SYR) in the weight range of 99-183 g were obtained from Charles River Laboratories (Wilmington, MA, USA). The animals were caged individually in polypropylene cages with pine shavings supplied for bedding and free access to tap water and Purina No. 5001 Rodent Chow (Ralston, St Louis, MO, USA).
Test substance formulation
Purified pseudoionone (amongst several retinoids and similar test substances) was dissolved in a small volume of reagent-grade acetone and solubilised in Tween 20 (Sigma Chemicals), with the final acetone concentration being 5%. The test substances were dispensed with as little air contact as possible and handled under yellow light, to minimise oxydation and photodegradation.
Test procedure
In the morning of day 8 after coition, the maternal weight was recorded and a single dose per animal of test substance or vehicle was given by oral gavage at a dose of 0.5 ml/100 g bw. The pseudoionone doses were 96 and 960 mg/kg bw. The animals were killed by carbon dioxide asphyxiation on day 14 after coition and weighed again. The pregnant uteri were excised by laparotomy, numbers of resorptions and dead foetuses were

recorded and living foetuses were examined under a binocular microscope. All foetuses were weighed and one-third (approximated) of each litter was fixed in Bouin's and subsequently sectioned sagittally. Two-thirds of each litter were fixed in 95% ethanol, eviscerated, cleared in 1% KOH and whole-stained with Alizarin Red S to show skeletal (mal)formation.
Statistics
The maternal weight change was calculated from the day of treatment to the day of termination. The final maternal weight values excluded the contribution of the litter as calculated by the total weight of all foetuses. Maternal weight change and mean litter bodyweights were analysed by one-way analysis of variance and the probability calculated by Newman-Keuls test. The number of resorptions for each test substance dose dose were compared the the vehicle control value by Mann-Whitney test. Abnormal litters were those containing one or more malformed foetuses or three or more resorbed implntation sites. The statistical significance of the numers of abnormal littes was analysed by the chi-square test with the Yates correction. Values were considered significantly different at the 95% confidence interval. The median effective dose for induction of terata and the embryonic LD50 were calculated for those retinoids associated with significant teratogenic response or elevated resorption rates.

Result: Administration of Tween20:acetone (95:5, v/v) alone was associated with a low incidence of embryonic and foetal death and malformation.
Single administration of pseudoionone resulted in the following observations:

Dose, mg/kg bw	96	960	0 (controls)
N treated	7	10	20
N litters	6	6	16
N abnormal litters	1	0	1
N implantation sites	67	79	208
N resorbed (%)	0 (0)	0 (0)	1 (0.5)
N foetuses examined	67	79	207
N abnormal live foetuses	0 (0)	0 (0)	1 (0.5)
N dead foetuses	1 (1.5)	0 (0)	1 (0.5)
Avg litter frequency of malformed foetuses	0	0	0.13
Avg foetal bodyweight, g	1.31±0.10	1.16±0.10	1.29±0.15
Avg maternal bodyweight change, g±SD	10.6±6.1	5.0±6.4*	10.2±6.1

Only the maternal bodyweight change at 960 mg/kg bw was significantly different from controls, but no single foetal or embryonic endpoint.

Test substance: Technical pseudoionone of approximately 65% purity by TLC was purchased from Pfaltz and Bauer (Atamford, CO, USA). Pseudoionone was purified by recycling preparative HPLC (Waters Ass., Model 500, Milford, MA, USA) on a silica column and eluted with HPLC-grade diethylether:hexane 90:10 (both Mallinckrodt). Purified pseudoionone was analysed at at purity of 98% by analytical HPLC and characterised by 1H-nuclear magnetic resonance spectroscopy.

Conclusion: A single oral administration of pseudoionone at 96 or 960 mg/kg bw to hamster females on day 8 of pregnancy did not result in any change or significant deviation from controls in embryonic or foetal parameters by day 14, when the study was terminated. There was only a significant effect on maternal

weight gain in the 960 mg/kg bw group, which was reduced compared to controls, however, the report states that "no other signs of intoxication were noted".
Based on this study there is no indication of foetal or embryotoxicity nor of teratogenicity due to single administration of pseudoionone.

Reliability: (2) valid with restrictions
Detailed study report with full data. Reliability 2.

Flag: Critical study for SIDS endpoint

12-FEB-2003 (106)

5.8.3 Toxicity to Reproduction, Other Studies

Type: other: Breeding (postnatal) toxicity
In Vitro/in vivo: In vivo
Species: rat
Strain: other: Wistar Crl: (WI) BR (outbred, SPF quality) **Sex:** male/female
Route of administration: other: gavage to dams
Exposure period: 21 days post partum
Frequency of treatment: once daily
Duration of test: 126 days
Doses: 0 (vehicle controls), 40, 120 and 360 mg/kg bw/d
Control Group: yes, concurrent vehicle

Method: other: OECD 415, One-generation reproductive toxicity
Year: 2002
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: For detailed methods, please see 5.8.1, Toxicity to Fertility.
Result: For general results, please refer to 5.8.1, Toxicity to Fertility.
Pups
The mean bodyweights of pups were signifcantly decreased during lactation at 120 and 360 mg/kg bw/d when compared to the control group. However, these decreases were only slight (between 90.6% and 95.6% of the concurrent control group) and all values were within the historical control data [given in the Appendix to the report], hence it was assumed that the statistically significant differences were probably obtained due to slightly higher concurrent control values. Therefore, this finding was not considered toxicologically relevant.
Breeding data
Breeding parameters were affected at 360 mg/kg bw/d. In the 360 group, postnatal loss during days 0-4 post partum was significantly increased. Due to this, the viability index was decreased in this group. The number of dead and living pups at first litter check, of living pups on day 4 post partum, of breeding losses during days 5-21 post partum, of living pups on day 21 post partum and the weaning index were similar for control and treated groups.

Test substance: Pseudoionone from Teranol AG, Lalden, batch no. UU02033826, purity 95.4% (area, GC).

Conclusion: Gavage treatment of male and female Wistar rats with pseudoionone at dose levels 0 (controls), 40, 120 and 360 mg/kg bw/d revealed breeding (post partum F1) toxicity at 360 mg/kg bw/d. Based on the results of this one-generation study, the breeding NOAEL was established at 120 mg/kg bw/d.

Reliability: (1) valid without restriction
Recent study according to OECD Guideline 415 under GLP, with full report and all individual data. Reliability 1.
Flag: Critical study for SIDS endpoint
12-FEB-2003 (9)

5.9 Specific Investigations

Endpoint: other: cytotoxicity, specifically growth rate of sarcoma cells
Result: significant inhibition of growth rate due to incubation with pseudoionone at relatively low concentration, full inhibition at 0.1 mM (= 19.2 mg/l), non-significant inhibition at 0.01 mM (= 1.92 mg/l)

Year: 1975
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Principle of the test
The dose-dependent inhibition of the cell division of cultured "ascites sarcoma BP8" cells by constitutents of tobacco and tobacco smoke, including pseudoionone, was tested.
Test system
"Ascites sarcoma BP8" stem cell cultures originating from inoculated C3H mice were grown in test tubes in Hams F10 medium sterilised by microfiltration (Millipore 0.45 µm), with foetal calf serum (15% w/w), penicillin (100 IU) and streptomycin (100 IU) added. The test tubes were gassed with sterilised air containing 5% carbon dioxide and capped air-tight to maintain a stable pH of approximately 7.3, as monitored with added phenol red maintained at pink to yellow. The cell cultures were re-inoculated to a cell density of 1000 cells/ml every fifth day. Cell densities were calculated with an electronic cell counter (Celloscope 401, Linson Instr. AB, Stockholm, Sweden) as this cell strain does not adhere to surfaces. For the test runs the cell suspension was diluted with sterile medium to an initial density of 4000 cells/ml.
Test performance
The compounds to be tested were dissolved in ethanol (10 µl) and/or dimethyl sulfoxide (10 µl) and added to the cell suspensions (3 ml), each in amounts to give the concentration listed below. Solvent only (10 µl) was added to the controls. After gassing and capping, the tubes were enclosed in a gassed (air, 5% CO2) and sealed plastic box to prevent errors due to pH changes caused by gas leaks in single tubes. All tubes were incubated in an oblique position at 37 °C for 48 hours. All test substances were run in duplicate, 8-10 controls were run per series of test compounds. Each series also comprised three or more internal standards in duplicate of substances known to have inhibitory effects, in most cases 2-aminonaphthalene, propranal [?type-setting error?], quinoline and isopentol. The growth rates of the duplicate cultures were calculated based on the cell counter values after 48 hours and compared to the mean of the controls. When a compound inhibited growth rate by 50% or more compared to controls, further experiments were performed at lower concentrations. The initial concentration for all substances was 1 mM, further

concentrations were 0.1, 0.01 and 0.001 mM. The endpoint of the test was inhibition of growth rate by less than 50%, meaning that inhibition may still have been statistically significant.

Remark: "Ascites sarcoma BP8" cells are stated not to possess the detoxication enzyme systems which enable many cells of the intact animal to convert foreign compounds to non-toxic excretable products or to reactive intermediates which may damage cell function.

Result: The normal growth rate for the controls was a doubling approximately every 24 hours.
Pseudoionone inhibited the growth rate by 100% at both 1 and 0.1 mM (192 and 19.2 mg/l, respectively) and by a statistically non-significant 9% at 0.01 mM (1.92 mg/l).

Test substance: Pseudoionone from an unstated source. It is stated that "[t]he purity of the compounds was tested by thin-layer chromatography, NMR or gas chromatography".
Another publication from the same group states: "All test substances including pseudoionone were checked for purity using TLC, GC and NMR. Compounds containing more the 3% impurities were purified using preparative LC, recrystallisation and distillation. The structures of the test compounds were confirmed by NMR."

Conclusion: Pseudoionone inhibited the cell division respectively growth rate of "ascites sarcoma BP8" cells completely at 0.1 mM (19.2 mg/l) and non-significantly at 0.01 mM (1.92 mg/l).
Significant cellular toxicity occurs at pseudoionone concentrations above 2 and below 20 mg/l.

Reliability: (2) valid with restrictions
Publication with description of test system and performance and with tabulated results including indication of statistical significance or not. Reliability 2.

03-JUN-2003 (79)

Endpoint: other: cytotoxicity, specifically ciliotoxicity on tracheal epithelium

Result: relatively rapid cessation of ciliary activity subsequent to short-term incubation with pseudoionone at relatively high concentration (5 mM = 962 mg/l)

Year: 1982

GLP: no data

Test substance: as prescribed by 1.1 - 1.4

Method: The effect of single compounds occurring in tobacco smoke on the function of ciliated tracheal epithelium was investigated.
Test system
Chicken tracheal organ cultures were prepared aseptically from 16- to 17-day-old chicken embryos. After dissection, the trachea was placed in minimum essential medium with Hank's salts (HMEM), HEPES (20 mM) and L-glutamine (2 mM). This medium was used throughout the investigation. The trachea was rinsed free of extratracheal tissues and medium was flushed through the trachea with a Pasteur pipette, in order to remove mucus and debris within the lumen. Subsequently, the trachea was cut transversely with a scalpel into rings of approximately 1 mm thickness. The rings from one trachea were transferred into a Petri dish containing the above medium and stored in a carbon-dioxide-gassed incubator (5% CO2 in air) at

37 °C and 80 %RH. Under these conditions the ciliary activity persisted for more than 4 weeks. However, the rings were normally used for experiments within 5-10 days of preparation.
Test procedure
Ciliary activity was observed at 37 °C by means of inverted microscopy using a magnification of x250. One single tracheal ring was placed in a Perspex testing chamber (volume = 3.1 ml) containing the medium admixed with an ethanol or dimethyl sulfoxide (DMSO) solution of the test respective compound. After addition of the tracheal ring, the chamber was closed to ambient air. The microscope was connected to a TV camera, a TV monitor and a videotape recorder allowing automated recording of ciliary activity. Activity was displayed continuously on the monitor during the whole exposure of maximally 60 minutes and recorded on videotape for 10 seconds every minute. The tape was then replayed and the time to complete cessation of ciliary activity was determined with a video-timer. Substance tests were performed in triplicate involving rings from different tracheal preparations.
It was ascertained before the main test that there was a high correlation between any the ciliary activity in any particular segement of the trachea and the whole circumference. The solvents were tested as negative controls and were found to be nontoxic to cilia at the concentration used in all experiments (1.6% v/v).
Test substance
Test compounds were dissolved in ethanol or DMSO to final concentrations of 5 mM test substance and 1.6% solvent in medium. Pseudoionone was dissolved in ethanol.

Result: Time to cessation of ciliary activity in the blank and solvent controls was >60 minutes, ie, longer than the time frame for testing.
Time to cessation of ciliary activity in the presence of 5 mM (= 962 mg/l) pseudoionone was 23 minutes. With pseudoionone, precipitates were noted in the test chambers, meaning that the actual concentration in the test medium may have been lower. However, as this was a screening of 300 different compounds, there were no substance-specific quantitative analytics in the media used.

Test substance: All test substances including pseudoionone were checked for purity using TLC, GC and NMR. Compounds containing more the 3% impurities were purified using preparative LC, recrystallisation and distillation. The structures of the test compounds were confirmed by NMR.

Conclusion: At a relatively high concentration of nominally 5 mM (0 = 962 mg/l), pseudoionone completely inhibited ciliary activity in excised embryonic chicken tracheal epithelium within 23 minutes.

Reliability: (2) valid with restrictions

Clear methods, data and quality control. Reliability 2.

12-FEB-2003 (77)

Endpoint: other: cytotoxicity, specifically plasma membrane toxicity in cultured human lung fibroblasts

Result: moderate (close to high) cell membrane damage subsequent to short-term incubation with pseudoionone at relatively high concentration (25 mM = 4800 mg/l)

Year: 1980

GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Human lung fibroblast cell cultures and radiolabelling
Human diploid embryonic lung fibroblasts (cell line MRC-5) were cultivated in Eagle's minimal essential medium (Flow Laboratories, Irvine, Scotland, UK) in polystyrene wells to aa cell density of 10E5 cells/cm2 (approximately 7*10E5 cells/well). The cells in confluent monolayers were labelled with [3H]uridine (NEN Chemicals, Frankfurt, Germany) to obtain a low-molecular-weight cytoplasmic marker, consisting of uridine nucleotides.
Test procedure
The cultures were exposed to the test substances to determine whether these would have a negative influence on the cellular plasma membrane resulting in leakage of the radiolabelled [3H]uridine into the medium. Labelled cultures were washed thrice with Hank's balanced salt solution (National Bacteriological Laboratory, Stockholm, Sweden) and subsequently incubated for 30 minutes at 37 °C with the respective test compound, one of which was pseudoionone, diluted to 25 mM in Tris-buffered saline (0.15 mM NaCl with 0.02 M tris-HCl from Merck, Darmstadt, Germany) at pH 7.0. Then, the medium containing leaked radioactive marker was removed and centrifuged (1000 g, 10 min, 4 °C) and the radioactivity in supernatant aliquots of 0.1 ml each was determined by scintillation counter.
As a positive control, maximum release of radioactivity was obtained by treating cells for 30 minutes with 0.06 M sodium borate buffer (pH 7.8) and scrapping with a "rubber policeman", a rubber-covered scraping or stirring rod. This treatment ruptured the cell membranes but left the nuclei intact. Non-treated cultures served as the negative, spontaneous background release control.
Calculation and characterisation of results
The relative leakage of radioactive marker in per cent was calculated by dividing the difference between experimental and spontaneous release by the difference between maximal and spontaneous release and multiplying with 100. Releases below 15% (twice the highest spontaneous rate) were considered insignificant, releases between 15% and 70% were termed moderate and releases above 70% were accepted as high.
Remark: The aim of this study was to specifically detect primary damage to the plasma membrane caused by tobacco and tobacco smoke components. Since cytoplasmic leakage may also arise as a secondary effect of general cytotoxic damage on prolonged exposure, it was necessary to use a short incubation time (30 min). As a consequence of this, a fairly high test substance concentration of 15 mM was selected to ensure that none of the genuinely membrane-damaging compounds escaped detection in this screening of 464 compounds.
Result: The spontaneous background release of radiomarker during 30 minutes at 37 °C was 3-7% of the maximal release.
Pseudoionone resulted in 68% relative release, which is in the upper range of the band termed "moderate" (15-70%).
Test substance: All test substances including pseudoionone were checked for purity using TLC, GC and NMR. Compounds containing more the 3% impurities were purified using preparative LC, recrystallisation and distillation. The structures of the test compounds were confirmed by NMR.

Conclusion: At a relatively high concentration of 25 mM (4800 mg/l), pseudoionone had a clear permeability-enhancing effect on cultured human lung fibroblasts. It is not possible to extrapolate this effect to lower concentrations or longer exposure, however.
Reliability: (2) valid with restrictions
Detailed publication with full methods, summary data and concise discussion. Reliability 2.
12-FEB-2003 (98)

Endpoint: other: competitive binding to retinol-binding protein
Species: human

Method: Retinol-binding protein (RBP) from the urine of patients suffering from "Itai-Itai" disease was purified by ammonium sulfate fractionation, gel filtration on Sephadex G-100 and finally chromatography on DEAE-cellulose. Details are given in the paper.
For the competitive binding experiment with pseudoionone, 0.1 ml of pseudoionone and 4.0 ml of standardised RBP solution (0.31 mg protein/ml) in Tris buffer were mixed, gently stirred for 1 minute and left to stand at room temperature for 30 minutes. To this mixture, 0.2 ml of a 0.35% retinol solution in n-heptane was added. Then the mixture was gently stirred for 10 minutes at room temperature and subsequently centrifuged at 3,000 rpm for 5 minutes. The aqueous layer containing the RBP fraction was analysed in a Hitachi EPS-3T spectrophotometer. The molar ratio of retinol to RBP was derived from the A330/A280 absorbance ratio. From this ratio the relative respectively competitive binding was derived. Details are given in the paper.
Remark: Retinol-binding protein (RBP) is a blood protein specific for vitamin A (retinol) transport. RBP is excreted in the urine of patients with certain diseases. RBP was purified from such urine and the relative binding to RBP of vitamin A derivatives and selected terpenes with structural similarities to parts of retinol, as well as a long-chained (C10) alcohol and a long-chained (C17) fatty acid, was determined.
Result: In comparison with the retinol standard, RBP pre-exposure to pseudoionone resulted in only 25% retinol binding, respectively 75% retinol-binding inhibition.
Test substance: Pseudoionone, purity not detailed, obtained from Takasago Perfume Co., Japan.
Conclusion: Pseudoionone had a high affinity to RBP. Among terpenoids, competitive binding was only higher in beta-ionone and beta-ionylidene acetic acid on one hand, both of which are characterised by a closed beta-ionone ring identical to the one in retinol, and by citral which like pseudoionone has a terminal respectively subterminal carbonyl group. In conclusion, RBP showed a high affinity for pseudoionone and pseudoionone is a potential inhibitor of RBP.
26-NOV-2003 (40)

6.1 Analytical Methods

Method: Polarography
Test substance: Pseudoionone

Result:

látka (substance)	slození roztoku (composition of solution)	E1	E2
pseudojonon	0.1 M Et4NI + 80% ethanol	-1.36	-1.79

(Et4NI possibly is tetraethyl ammonium iodide)

Conclusion: Basic conditions and E1/E2 values for the polarographic indentification and quantification of pseudojonon are given in highly abstracted form.

Reliability: (2) valid with restrictions
In spite of the extreme briefness of the presentation, the data are from the chemist who developed polarography and later received the Nobel Prize for his work (J Heyrovský). Hence reliability is regarded as high.

15-JAN-2003 (42)

Method: Gas chromatography
Test substance: Pseudoionone

Method:

GC type	Beckman GC-2/FID	Beckman Thermotrac
Column	DEGS	SE-30
Carrier gas	N2	H2
Flow		3 l/h
Pressure	1.5 atm	
Temperature	160 °C	120-200 °C/15 min
Relative retention values:		
cis-Pseudoionone	10.0	4.53
trans-Pseudoionone	13.9	4.97

04-JUN-2003 (66)

Method: Thin-layer chromatography
Test substance: Pseudoionone

Method:

Plates	glass, 10X20 cm	glass, 10X20 cm
Layer	250 um	250 um
Sorbent	KieselgelG (Merck)	KieselgelG + 10% AgNO3
Mobile phase	benzene:ethyl acetate 9:1	benzene:ethyl acetate 8:2
Detection	UV light SbCl3 in chloroform	UV light SbCl3 in chloroform
Relative retention values:		
cis-Pseudoionone	0.56	0.57
trans-Pseudoionone	0.56	0.57

04-JUN-2003 (66)

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