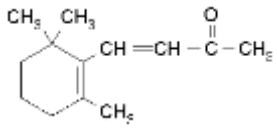
Ionone, beta-

Ingredient synonym names

4-(2,6,6-Trimethylcyclohex-l-enyl)-but-3-en-2-one
beta- Irisone
beta-Cyclocitrylidene acetone
trans-beta-Ionone

IDENTIFIER DETAILS

Ingredient chemical structure



CAS Number	FEMA Number	Additive Number	Ingredient EC Number
79-77-6	2595	-	201-224-3

CAS Additional Number	FL Number	CoE Number
14901-07-6	07.008	142

Chemical formula C13H20O

Ingredient CLP Classification

Ingredient REACH Registration Number

-

Acute Oral Toxicity	Eye Damage/Irritation	Carcinogenity
0	0	0
Acute Dermal Toxicity	**Respiratory Sensitisation**	**Reproductive Toxicity**
0	0	0
Acute Inhalation Toxicity	**Skin Sensitisation**	**Aspiration Toxicity**
0	0	0
Skin Corrosive/Irritant	**Mutagenicity/ Genotoxicity**	**Specific Target Organ Toxicity**
0	0	0

SPECIFICATIONS

Melting Point	Boiling Point
No data provided.	229°C

STATUS IN FOOD AND DRUG LAWS

Ionone, beta-

Acceptable Daily Intake (ADI, mg/kg)	0 - 0.1mg/kg
Acceptable Daily Intake (ADI) comments	JECFA (1984) Maintained in 1998
FDA Status	172.515: Synthetic flavouring substances and adjuvants

CoE limits - Beverages (mg/kg)	No data provided.	CoE limits - Food (mg/kg)	No data provided.	CoE limits - Exceptions (mg/kg)	No data provided.

HUMAN EXPOSURE

Ingredient Natural Occurence (if applicable)

β-Ionone is reportedly found in raspberry, apricot, orange juice, grapes, melon, fenugreek, tamarind and clary sage and in the distillate from flowers of Boronia megatisma Nees [Fenaroli, 2005]. It is synthesised by condensing citral with acetone to form pseudoionone, which is then cyclized by acid type reagents [Fenaroli, 2005]. Main natural occurrence in food [mg/kg]: Raspberry: 0.05-1.3; carrot: 8.96; cognac: 0.4; green tea: 0.4-6.4; and roasted almond: 0.5 [COE No.142]. β-Ionone is a mixed acylic aliphatic ketone, regarded as important for flavour of tea, with levels in black tea of about 1.3-4.4 mg/kg [IARC, 1991].

References - Ingredient Natural Occurence

Fenaroli (2005) Handbook of Flavour Ingredients. 5th Edition. CRC press.

IARC (1991). Monographs on the Evaluation of Carcinogenic Risks to Humans: Tea. 51: 207

Ingredient Reported Uses

β-Ionone is reportedly used at maximum levels in baked goods at 10.67ppm, frozen dairy at 6.64 ppm, soft candy at 12.03 ppm, gelatin pudding at 6.65 ppm, non-alcoholic beverages at 3.21 ppm, alcoholic beverages at 0.40 ppm, hard candy at 3.52 ppm, and chewing gum at 275.4 ppm. Individual consumption of 0.001709 mg/kg/day was reported [Fenaroli, 2005].

References - Ingredient Reported Uses

Fenaroli (2005) Handbook of Flavour Ingredients. 5th Edition. CRC press.

TOXICITY DATA

In Vivo Data

Acute Toxicity Data

LD50s

2277 mg/kg, Mouse, Intraperitoneal

Sporn et al., 1963

4590 mg/kg, Rat, Oral

Jenner et al., 1964

2605 mg/kg, Mouse, Sub-cutaneous

ESIS, 2004

When the test material was a mixture of 60 % α-ionone and 40 % β-ionone, the rat oral LD50 was 4,590 mg/kg [JECFA, 1998].

References

ESIS (2004). As obtained from http://ecb.jrc.it/

JECFA, (1998). Safety evaluation of certain food additives and contaminants. Prepared by the 49th meeting of the Joint FAO/WHO Expert Committee on Food Additives.

Jenner et al., (1964). Food flavourings and compounds of related structure. I Acute oral toxicity. Food & Cosmetic Toxicology. 2: 237.

Sporn et al., (1963). The toxicity of butyl acetate, methyl naphthyl ketone and ionone. Iginea 12: 437.

In Vivo Carcinogenicity/Mutagenicity

No data identified.

References - In Vivo Carcinogenicity/Mutagenicity

No data identified.

Dermal Toxicity

The OECD SIDS report for β-ionone concluded that in studies conducted according to OECD test guidelines and under GLP conditions, β-ionone was not irritating to the skin of rabbits after semi-occlusive application for 4 hours and only slightly irritating to the eyes. After a 24-hours exposure under occlusive conditions, a slight irritation of the skin was observed in rabbits. A limited human patch test did not reveal a potential for skin irritation when a not further specified mixture of α- and β -ionone was applied undiluted to the skin of volunteers. A limited Guinea pig maximization test found no evidence that β -ionone is a dermal sensitizer. According to secondary sources, ionone (a not further specified mixture of α - and β -ionone) was negative in an open epicutaneous test with Guinea pigs as well as in a human maximization test with a product containing 97.5 % α -ionone and 2.5 % β-ionone [OECD SIDS, 2004].

Three rabbits received a single dermal application of 5% β-ionone in diethyl phthalate on abraded and intact skin exhibited negative results up to 72 hrs. Application of 5% β-ionone in diethyl phthalate resulted in very slight to well defined erythema on abraded and intact skin of 2 rabbits at 24 h, which cleared by 72 h. There was very slight edema on abraded and intact skin of 2 rabbits at 24 h, which cleared by 72 h. (Lalko et al, 2007).

References - Dermal Toxicity

OECD SIDS report on β-Ionone, (2004), UNEP publications,
http://www.inchem.org/documents/sids/sids/79776.pdf

Lalko J, Lapczynski A, McGinty D, Bhatia S, Letizia CS, Api AM (2007) Fragrance material review on beta-

ionone. Food and Chemical Toxicology 45: S241-S247.

Reproductive/ Developmental Toxicity

In a well-conducted 90 days study in rats according to OECD TG 408 with administration of the test substance in the diet, β-ionone did not have the potential to damage the reproductive organs up to the highest tested concentration of 10 000 ppm (720 and 801 mg/kg bw/day for males and females) [OECD SIDS, 2004].

The OECD SIDS report for β-ionone described a prenatal developmental study with gavage application of β-ionone conducted in Wistar rats. The study conformed to GLP and developmental toxicity study guildlines (OECD TG 414). The no observed adverse effect level (NOAEL) for maternal toxicity was 100 mg/kg bw/day. The NOAEL for prenatal developmental toxicity could be fixed at the highest tested dose (400 mg/kg bw/day). The test substance had no influence on gestational parameters and induced no adverse signs of developmental toxicity and in particular no indications of teratogenic effects up to and including the highest dose level were observed [OECD SIDS, 2004].

The reproductive and embryotoxic effect of β-ionone was evaluated in pregnant Wistar rats. Animals were administered a single dose of β-ionone dissolved in corn oil by gavage on pregnancy day 11, at dose levels of 250, 500, 750 and 1000 mg/kg. Animals were weighed on days 0, 11, and 21 of pregnancy, and sacrificed on day 21. At sacrifice, the uterus was weighed with its contents and the number of living and dead fetuses, implantation sites, and resorptions were recorded. In addition, the living fetuses were weighed and examined for externally visible anomalies with scoring from 0 (absence) to 4 (severe). With 250, 500 and 750 mg/kg, no effects were produced. Compared to the untreated controls, the uterus weight, the ratio of resorptions per implantations and the percentage of resorptions per implantation per litter were substantially increased, and the ratio of live fetuses per implantations per litter was drastically decreased with 1000 mg/kg (Gomes-Carneiro et al., 2003). (Lalko, 2007).

β-ionone (BIO) is used in fragrances, toiletries and non-cosmetic products, and as a flavor food additive. Notwithstanding the widespread human exposure, there are limited data on the reproductive toxicity of BIO. This study evaluated the developmental toxicity of BIO (0, 125, 250, 500 and 1000 mg/kg body weight/day) given orally to rats on days 6-15 of gestation (GD6-15). C-section was on GD21 and implantations, living and dead fetuses and resorptions were recorded. Fetuses were weighed, and examined for external abnormalities and skeleton and visceral anomalies. The embryotoxicity of a single oral dose of BIO (1000 mg/kg body wt) given on GD11 was evaluated as well. At the highest dose, BIO reduced weight gain and produced chromodacryorrhea and other signs of toxicity. BIO did not increase the frequency of malformations nor did it retard fetal growth. Nonetheless, BIO decreased the pregnancy rate in the group of females exposed on GD6-15, and increased the resorption rate in those treated on GD11 only. In conclusion, except for a higher embryolethality at a maternally toxic dose, BIO caused no embryotoxic effect over the dose range tested and the study NOAEL for maternal and developmental toxicity was 500 mg of BIO/ kg of body weight/day. [Pinto, F. C., et al. (2018)]

References - Reproductive/ Developmental Toxicity

OECD SIDS report on β-Ionone, (2004), UNEP publications, http://www.inchem.org/documents/sids/sids/79776.pdf

Lalko J (2007). Fragrance material review on β-ionone. Food and Chemical Toxicology Volume 45, Issue 1, Supplement 1, Pages S241–S247

Pinto, F. C., De-Carvalho, R. R., De-Oliveira, A. C. A., Delgado, I. F., & Paumgartten, F. J. (2018). Study on the developmental toxicity of β-ionone in the rat. Regulatory Toxicology and Pharmacology, 97, 110-119.

Inhalation Toxicity

No data identified.

References - Inhalation Toxicity

No data identified.

Cardiac Toxicity

No data identified.

References - Cardiac Toxicity

No data identified.

Addictive Data

No data identified.

References - Addictive Data

No data identified.

Behavioral data

No data identified.

References - Behavioral data

No data identified.

In Vivo - Other Relevant Studies

β-Ionone [and other isoprenoids] inhibit 3-hydroxy-3-methylglutaryl coenzyme A reductase activity, an important enzyme for cholesterol production. The author postulated that a diet rich in β-ionone could have a protective effect against cancer and cardiovascular disease.
β-Ionone had a slight positive effect on the motility of mice when they were exposed by inhalation. However, when the mice had been over agitated by injection of caffeine, β-ionone had a sedative effect [Buchbauer et al., 1993].

β-Ionone is metabolised by carbonyl reduction, hydroxylation of the alicyclic ring and glucuronic acid conjugation of metabolites with alcohol groups (JECFA 1999). In the rabbit administration of β-Ionone at approximately 1000 mg/kg/bw day, the following metabolites were found in the urine 3-oxo-β-ionone; 3-oxo-β-ionol; 3-hydroxy-β-ionol and unchanged -β-ionone [Ide et al., 1970].

When melanomas were established in mice prior to treatment with 2µmol/kg β-ionone, the host survival increased. The authors suggest the presence of isoprenoids in the diet [from fruit, vegetables and cereal grains] could explain the anticarcinogenic action of plant-based diets [He et al., 1997].

Secondary products of plant mevalonate metabolism suppress the synthesis of mevalonate, the rate limiting intermediate in the biosynthesis of isoprenoid derived compounds that stimulate cell proliferation. They suppress the activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase, exhibit low toxicity and have proven their efficacy in animal cancer chemoprevention studies [Jung et al., 1998].

β-Ionone dosed at 600 mg/kg/day to male BALB/c mice significantly induced P450 2B specific pentoresorufin O-dethylase and benzyloxyresorufin O-debenzylase, however this dosage was not hepatotoxic. β-Ionone also induced other forms of P450 [Jeong et al., 1999].

Jeong et al., (1999), reported that pretreatment of male BALB\c mice with β-Ionone at 600 mg/kg 72 and 48 hour prior to the intraperitoneal administration of thioacetmide at either 100 or 200 mg/kg. β-Ionone significantly induces cytochrome P450 2B-specific pentoresorufin O- depentylase and benzyl oxyresorufin O-debenzylase activities and potentiated thioacetmide induced hepatotoxicity. The pre-treatment with beta-ionone was not hepatotoxic to the mice [Jeong et al., 1999].

Pentoxyresorufin O-dethylase a selective marker for CYP2B1 was found to be inhibited by β-Ionone at IC50 as low as 0.03 µmol and was also a week inhibitor of ethoxyresorufin O-deethylase (EROD) a selective marker for CYP1A1. beta-Ionone may therefore interfere with xenobiotic substrates that are substrates for this isoenzyme [De-Oliveira et al., 1999].

Five Wistar rats treated i.p for a period of four-days with β-Ionone, (1 g/kg bw) showed a non-statistically significant increase in CYP2A3 mRNA in the lung, (Total RNA was extracted from a series of tissues). The data from this particular study revealed that rat CYP2A3 is constitutively expressed in extrahepatic tissues and its regulation occurred through an essentially tissue specific mechanism [Robottom-Ferreira et al., 2003].

Liu et al. (2010) examined the chemopreventive effects of varied doses of dietary beta -ionone on the development and growth of DMBA-induced rat mammary tumors as well as plasma antioxidant status. Beta -ionone treatment groups were given 9, 18, and 36mmol/kg in the AIN76A diet starting 2 weeks prior to DMBA administration and continuing for the 24th week. Results showed that tumour incidence was dose dependently reduced by 35.4, 68.3, and 87.8 %, respectively, compared to the positive control. Tumour sizes were dose dependently smaller, and tumour weight was less in each group, each rat, and each tumour compared to the positive control ($P < 0.05$). A significant decrease in lipid peroxidation was observed in the tumour-induced rats treated with dietary beta-ionone, whereas the plasma activities of antioxidant enzymes such as glutathione peroxidase, glutathione reductase, superoxide dismutase, and the nonenzymatic antioxidant glutathione were increased in the beta -ionone treated rats when compared to control. The levels of catalase and lactate dehydrogenase were remarkably decreased in the beta -ionone treated groups compared to the positive control group. These results suggest that dietary beta -ionone has biologically relevant antioxidant activity and plays a chemopreventive role against DMBA induced mammary gland tumours [Liu et al. 2010].

With the test material as a mixture of 60 % α-ionone and 40 % β-ionone at 0, 0.1, 0.25, and 1 % in the diet, it reportedly caused a dose dependent moderate swelling of liver parenchyma in rats. Similarly at doses as low as 1000 ppm [~ 50 mg/kg] although no adverse effects were observed on growth, appearance, food intake, haematology, body weight, organ weights or macroscopic organ appearance, swelling of hepatic parenchyma was observed on microscopic examination [JECFA, 1998].
β-Ionone, when tested in a 90 day feeding study in rats, resulted in a no untoward effect level [NUEL] of 10 mg/kg. At 100 mg/kg changes in renal function were observed [IARC, 1991].

A rat 90-day feeding study in which rats were fed 10 and 100 mg/kg reported a NOAEL of 10 mg/kg bw. Similarly another 90-day feeding study reported a NOAEL of 11.6 mg/kg bw, [ESIS, 2004].

An OECD SIDS report for β-ionone describes an animal study where β-ionone was added to the diet of Wistar rats. ß -ionone was administered over a period of 90 days according to OECD TG 408 at dietary concentrations of 100, 1000 and 10 000 ppm (7 and 8 mg/kg bw/day, 72 and 83 mg/kg bw/day or 720 and 801 mg/kg bw/day for males and females) to rats. This led to signs of general systemic toxicity at the high and mid dose. Target organs were liver, kidneys and thyroid glands. The liver findings in both sexes and the increased kidney weights in high dose females were indicative of adaptive and most likely reversible processes with the aim to increase the metabolizing and/or excretory capacity of these organs. The findings in males with respect to kidneys as well as kidney relevant parameters should be seen in the light of high amounts of alpha2u-globulin in these animals. The occurrence of alpha2u-globulin was confirmed by immunohistochemical examination. The accumulation of this protein appears to be a unique feature of male rats and is not known to occur in other species, including man. No

signs of neurotoxicity were observed during functional observational battery as well as measurement of motor activity performed towards the end of the administration period. Thus, the no-observed-effect-level (NOEL) under the conditions of the present study was 100 ppm for both sexes (about 7 and 8 mg/kg bw/day for males and females) based on adaptive liver effects in both sexes and minor urine findings in males at 1000 ppm which correspond to a dosage of 72 and 83 mg/kg bw/day for males and females (no-observed-adverse-effect-level, NOAEL). The lowest-observed-adverse-effect-level (LOAEL) was found at 10,000 ppm (720 and 801 mg/kg bw/day for males and females) due to liver, kidney and thyroid findings in both sexes [OECD SIDS, 2004].

Eye irritation in rabbits was evaluated according to Draize test. There were no adverse effects observed by 4-(2,6,6-trimethylcyclohex-1-ene-1-yl)-but-3-ene-2-one indicating that 4-(2,6,6-trimethylcyclohex-1-ene-1-yl)-but-3-ene-2-one is not irritating to eye of rabbit.
Executive summary: Eye irritation in rabbits was evaluated according to Draize test. There were no adverse effects observed on application of neat 4-(2,6,6-trimethylcyclohex-1-ene-1-yl)-but-3-ene-2-one. The overall result indicate that 4-(2,6,6-trimethylcyclohex-1-ene-1-yl)-but-3-ene-2-one is not irritating to eye of rabbit. (Lalko et al, 2007).

Effects of beta-ionone on the expression of other P450 isozymes and NADPH-P450 reductase were further investigated in Sprague Dawley rats. Administration of beta-ionone subcutaneously 72 and 48 h before sacrificing the animals not only significantly induced the liver microsomal activities of P450-associated enzymes and NADPH-P450 reductase, but also clearly increased in the level of P450 1A1/2, P450 2C, and NADPH-P450 reductase proteins. The induction of P450 1A1/2 and 2C by beta-ionone was much greater in male than in female as measured by western immunoblotting. Reverse transcriptase-polymerase chain reactions showed that, in addition to P450 2B1 and 2B2 mRNAs, P450 1A2, 2C6 and NADPH-P450 reductase mRNAs were increased when beta-ionone was administered. Our previous and present results indicated that beta-ionone may induce several P450s and NADPH-P450 reductase by the accumulation of their corresponding mRNAs. (Jeong et al, 1998).

References - In Vivo - Other Relevant Studies

Buchbauer et al., (1993). Fragrance compounds and essential oils with sedative effects upon inhalation. Journal of Pharmaceutical Science. 82: 660.

De-Oliveira et al., (1999). In Vitro inhibition of liver monoxygenases by β-ionone, 1,8 –cineol, (-) menthol and terpineol. Toxicology 135: 33-41.

ESIS (2004).

He et al., (1997). Isoprenoids suppress the growth of murine B16 melanomas in vitro and in vivo. Journal of Nutrition. 127: 668.

Ide et al., (1970). Metabolism of β ionone. Isolation, Characterisation and identification of the metabolites in the urine of rabbits. Biochemical Journal. 119: 281-287.

IARC (1991). Monographs on the Evaluation of Carcinogenic Risks to Humans: Tea. 51: 207.

JECFA, (1998). Safety evaluation of certain food additives and contaminants. Prepared by the 49th meeting of the Joint FAO/WHO Expert Committee on Food Additives.

Jung et al., (1998). Synthesis and Biological activity of beta-ionone derived alcohols for cancer chemoprevention. Anticancer Research. 18: 189-192.

Jeong et al., (1999). Pretreatment of male BALB/c mice with beta-ionone potentates thioacetamide–induced hepatotoxicity. Toxicology Letters. 105(1): 39-46.

Liu JR, Dong HW, Sun XR, Wang Q, Sun WG, Parry JW, Liu Q, Han XH, Sun CH, Chen BQ, Yang BF. (2010) Effects of beta-ionone on mammary carcinogenesis and antioxidant status in rats treated with DMBA. Nutr Cancer; 62: 58-65.

OECD SIDS report on β-Ionone, (2004), UNEP publications.

Robottom-Ferreira et al., (2003). Expression of CYP2A3 mRNA and its regulation by 3-methylcholanthrene, pyrazole, and beta-ionone in rat tissues. Braz J Med Biol Res. 36(7): 839-44.

Lalko J, Lapczynski A, McGinty D, Bhatia S, Letizia CS, Api AM (2007) Fragrance material review on β-ionone. Food and Chemical Toxicology 45: S241-S247.

Jeong TC et al (1998). Effects of beta-ionone on the expression of cytochrome P450s and NADPH-cytochrome P450 reductase in Sprague Dawley rats. Chem Biol Interact. 114(1-2):97-107

In Vitro Data

In Vitro Carcinogenicity/Mutagenicity

An EFSA report from 2014, reported data from an unpublished report submitted by EFSA to FLAVIS (Stone, 2011). Beta-ionone [FL-no: 07.008] did not induce micronuclei up to toxic concentrations when assayed in cultured human peripheral lymphocytes for 3 + 21 hours in the absence and presence of S9-mix or when incubated for 24 + 0 hours in the absence of S9-mix [EFSA, 2014].

β-Ionone had some in vitro activity against a murine melanoma cell line [B16], with an IC50 of 140 ± 23 μm/litre [He et al., 1997].

β-Ionone has been shown to be cytotoxic to the BT-20 breast carcinoma cells with an IC50 of 3.23 μg/ml and also cytotoxic to HeLa cells with an IC50 of 2.96 μg/ml, Kubo et al., [1995].

β-Ionone has been shown not to be mutagenic in the Ames test, both in the absence and presence of an S-9 fraction, at concentration from 3 μmol/plate to 180 mg/plate in strains TA98, TA100, TA1535, TA1537 [Florin et al, 1980; Mortlemans et al, 1986].

The Salmonella/microsome assay was used to assess the mutagenicity and antimutagenicity of β-Ionone in S.Typhimurium strains TA100, TA98, TA97a and TA1535 with / without S9 metabolic activation (MA) initially using the plate incorporation procedure followed by the pre-incubation test for confirmation. Both assays concluded that beta-ionone was non-mutagenic in vitro using concentrations of β-Ionone (0-500μg/plate – with S9 MA) and (0-5000μg/plate – without S9 MA). Antimutagenicity was also assessed using the plate incorporation method with different non-toxic doses of β-Ionone (0-200μg/plate) against one or more non-toxic doses of a) Directing acting mutagens such as sodium azide, 4-nitroquinoline-N-oxide and 2-Nitrofluorene and b) Indirect acting mutagens such as cyclophosphamide, benzo-(α)-pyrene, 2-aminofluorene and aflatoxin B1. No antimutagenic effects were observed in relation to direct acting mutagens, B-(α)-P and 2-AF. However, mutagenic effects of AFB1 and CP were dose dependently antagonised in the presence of β-Ionone possibly via inhibition of CYP2B enzymes within the liver [Gomes-Carneiro et al., 2006].

β-Ionone at concentrations of 25, 50, 100 and 200 mumol/L was reported to inhibit cell proliferation, cellular mitosis, cell clone formatting and DNA synthesis in human mammary cancer cells, (MCF-7), (IC50 reported to be 104 mumol/L, [Liu et al., 2004a]. Similarly, at these concentrations beta-ionone inhibited cell proliferation and induced apoptosis in the gastric adenocarcinoma cell line SGC-7901 [Liu et al., 2004b]. Further studies also revealed that beta-ionone could up-regulate the expression of TIMP-1 and TIMP-2 (metalloproteinases)

expression, and ‘may influence metastasis of cancer’ [Liu et al., 2004c].

Human breast cancer cells (MBA-MB 435) containing a non-receptor of estrogen (Er), induced by β-ionone were treated with 25, 50, 100 and 200 micromol/L of β-ionone. With increasing concentration cell proliferation, cellular mitosis, clone formatting and DNA synthesis was inhibited (inhibition rates, 45.67 %, 71.24 %, 81.53 % and 84.93 % respectively). Reduced expression of PCNA protein (related to the cell cycle), ERK, MEK-1 protein expression coupled with the promotion of JNK and MKP-1 proteins related to the MAPK pathway was observed indicating that β-ionone may inhibit MDA-MB 435 cell proliferation by regulating MAPKs pathway (Lui et al., 2005).

Asokkumar et al., (2012) aimed to evaluate the therapeutic efficacy of beta-ionone (ION), a precursor for carotenoids against benzo(a)pyrene [B(a)P]-induced lung carcinogenesis. B(a)P (50 mg/kg body weight, orally twice a week for 4 successive weeks)-induced lung cancer in mice was assessed both in tissue and serum in terms of increase LPO and tissue marker enzymes, such as aryl hydrocarbon hydroxylase, γ-glutamyl transpeptidase, 5'-nucleotidase, and lactate dehydrogenase, and serum tumor markers such as carcinoembryonic antigen and neuron-specific enolase with concordant decrease in activities of tissue enzymic and non-enzymic antioxidants were observed on the treatment of ION (60 mg/kg body weight, orally twice a week for 16 weeks) significantly attenuated LPO and restored all cancer marker enzymes and antioxidants levels to near normal, which indicates the anticancer effect of ION. This was further confirmed by histological staining of argyrophilic nucleolar organizer region and histopathological analysis of lung tissue, immunohistochemical and immunoblot analysis of proliferating cell nuclear antigen. Overall findings suggested that the ION effectively ameliorated the lung carcinogenesis, which is attributed to the antiproliferative and antioxidant potential through free radical scavenging property. [Asokkumar et al., 2012].

β-Ionone is an end ring analogue of β-carotenoid which has been shown to possess potent anti-proliferative activity both in vitro and in vivo. To investigate the possible inhibitory effects of β-ionone, we studied cell growth characteristics, DNA synthesis, cell cycle progression, as well as mitogen-activated protein kinases (MAPKs) pathways in the human gastric adenocarcinoma cancer cell line (SGC-7901). Our results show that cell growth and DNA synthesis were inhibited, and the cell cycle was arrested at the G0/G1 phase in a dose-dependent manner in cells treated with β-ionone (25, 50, 100 and 200 μmol/L) for 24 h. We found that the β-ionone significantly decreased the extracellular signal-regulated kinase protein expression and significantly increased the levels of p38 and Jun-amino-terminal kinase protein expression ($P < 0.01$). β-Ionone also inhibited cell cycle-related proteins of Cdk4, Cyclin B1, D1 and increased p27 protein expression in SGC-7901 cells. These results suggested that the cell cycle arrest observed may be regulated through a MAPK pathway by transcriptional down-regulation of cell cycle proteins. These results demonstrate potent ability of β-ionone to arrest cell cycle of SGC-7901 cells and decrease proliferation [Dong et al., 2013].

β-Ionone, a cyclic sesquiterpene and an end-ring analog of β-carotene, induced concentration-dependent inhibition of the proliferation of human DU145 (IC50 = 210 μmol/L) and LNCaP (IC50 = 130 μmol/L) prostate carcinoma cells and PC-3 prostate adenocarcinoma cells (IC50 = 130 μmol/L). Concomitantly, β-ionone-induced apoptosis and cell cycle arrest at the G1 phase in DU145 and PC-3 cells were shown by fluorescence microscopy, flow cytometry, and TUNEL reaction, and downregulation of cyclin-dependent kinase 4 (Cdk4) and cyclin D1 proteins. Growth suppression was accompanied by β-ionone-induced downregulation of reductase protein. A blend of β-ionone (150 μmol/L) and trans, trans-farnesol (25 μmol/L), an acyclic sesquiterpene that putatively initiates the degradation of reductase, suppressed the net growth of DU145 cells by 73%, an impact exceeding the sum of those of β-ionone (36%) and farnesol (22%), suggesting a synergistic effect. β-ionone, individually or in combination with other HMG CoA reductase suppressors, may have potential in prostate cancer chemoprevention and/or therapy [Jones et al., 2013].

Based on the prediction for in-vitro mammalian chromosome aberration test on Chinese hamster Overy (CHO) it was estimated that 4-(2,6,6-trimethylcyclohex-1-ene-1-yl)-but-3-ene-2-one does not exhibit positive chromosomal effect. (Danish EPA, 2012).

Beta-ionone, an end-ring analogue of beta-carotenoid, which is a constituent of vegetables and fruits, has been analyzed for colon cancer chemoprevention and treatment. beta-Ionone induced cell growth inhibition and apoptosis in human colon cancer HCT116 cell line. We tested the in vivo chemopreventive efficacy in rat colon carcinogenesis model using aberrant crypt foci (ACF) as endpoint marker. HCT116 cells treated with subtoxic concentrations of beta-ionone resulted dose-dependent cell growth suppression with G1-S-phase growth arrest and significant induction of apoptosis. beta-Ionone up-regulated expression of retinoid X receptor-alpha mRNA dose-dependently in HCT116 cells. To evaluate inhibitory properties of beta-ionone on colonic ACF, 7-week-old male F344 rats were fed experimental diets containing 0%, 0.1%, or 0.2% beta-ionone. After 1 week, rats received s.c. injections of azoxymethane, 15 mg/kg body weight, once weekly for 2 weeks. Rats were continued on respective experimental diets and sacrificed 8 weeks after the azoxymethane treatment. Colons were evaluated histopathologically for ACF. Administration of dietary 0.1% and 0.2% beta-ionone significantly suppressed total colonic ACF formation up to 34% to 38% ($P<0.0002$ to $P<0.0009$), respectively, when compared with control group. Importantly, rats fed beta-ionone showed >55% inhibition ($P<0.0001$) of foci containing four or more aberrant crypts. Results from in vitro and in vivo bioassay clearly suggest that beta-ionone could be further developed for prevention and treatment of colon cancer. (Janakiram et al, 2008).

Recent chemopreventive studies from our group showed that dietary beta -ionone inhibited 7,12-dimethylbenz(a)anthracene (DMBA)-induced mammary carcinogenesis by the inhibition of cell proliferation and apoptosis initiation. In this study, we examined the chemopreventive effects of varied doses of dietary beta -ionone on the development and growth of DMBA-induced rat mammary tumors as well as plasma antioxidant status. beta -ionone treatment groups were given 9, 18, and 36 mmol/kg in the AIN76A diet starting 2 wk prior to DMBA administration and continuing for the 24 wk. Results showed that tumor incidence was dose dependently reduced by 35.4, 68.3, and 87.8%, respectively, compared to the positive control. Tumor sizes were dose dependently smaller, and tumor weight was less in each group, each rat, and each tumor compared to the positive control ($P < 0.05$). A significant decrease in lipid peroxidation was observed in the tumor-induced rats treated with dietary beta -ionone, whereas the plasma activities of antioxidant enzymes such as glutathione peroxidase, glutathione reductase, superoxide dismutase, and the nonenzymatic antioxidant glutathione were increased in the beta -ionone treated rats when compared to control. The levels of catalase and lactate dehydrogenase were remarkably decreased in the beta -ionone treated groups compared to the positive control group. These results suggest that dietary beta -ionone has biologically relevant antioxidant activity and plays a chemopreventive role against DMBA induced mammary gland tumor. (Liu, 2010).

Beta-ionone is an aroma compound found in the Rosaceae family. Some evidence supported that beta-ionone has a great potential for cancer prevention. The results demonstrated that beta-ionone has anti-proliferative and apoptotic effects on K562 cells, and in the future may be used in the treatment of some leukemia sub-types. (Faezizadeh et al, 2015).

β-Ionone is an end-ring analog of β-carotenoids which widely distributed in fruit and vegetables. Recent studies have demonstrated anti-proliferative, anti-metastatic and apoptosis induction properties of β-ionone in vitro and in vivo. Also, the studies have focused on investigating the β-ionone action on different types of malignant cells and the possible mechanisms of action. Moreover, the quest of new synthetic β-ionone-based compounds possessing anti-proliferative, anti-metastatic and apoptosis induction activities may enable the discovery of compounds which can be used in combination regimes thus overcoming tumor resistance to conventional anticancer agents. These new agents will also be useful for targeting distinct signaling pathways, to activate selectively mechanisms for apoptosis in cancer cells but devoid of undesirable side effects. In this paper, we reviewed the potentialities of β-ionone and related compounds in cancer prevention and chemotherapy. (Ansari et al, 2016).

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In Vitro - Other Relevant Studies

beta-Ionone is a constituent of vegetables and fruits, and can induce apoptosis in some types of malignant cells. However, the mechanism of apoptosis in osteosarcoma (U2os) cells is currently unclear. In this study, we determined whether beta-ionone can induce apoptosis in U2os cells in vitro and which signal pathway(s) is involved. We found that beta-ionone inhibited cell proliferation in U2os cells in a concentration- and time-dependent manner and caused cell cycle arrest at the G1-S phase. TUNEL assay, DNA ladder and assessment of Caspase 3 activity showed that apoptosis was the determinant in the effects of beta-ionone. Furthermore, Expression of the p53 protein increased in a concentration-dependent and time-dependent manner according to immunocytochemistry and immunoblotting after beta-ionone treatment. In addition, beta-ionone upregulated Bax protein and downregulated Bcl2 protein which led to Bax translocation and cytochrome c release, subsequently activated Caspase 3, thus resulting in apoptosis. In summary, these data suggested that beta-ionone induced apoptosis in a concentration-dependent manner in U2os cells via a p53-dependent mitochondrial pathway. (Zhu, 2010).

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Emissions and Associated Toxicity Data

Carmines (2002), Rustemeier et al., (2002), Roemer et al., (2002) and Vanscheeuwijck et al., (2002) reported on a testing program designed to evaluate the potential effects of 333 ingredients added to typical commercial blended test cigarettes on selected biological and chemical endpoints. The studies performed included a bacterial mutagenicity screen [Ames assay] a mammalian cell cytotoxicity assay [neutral red uptake], determination of smoke chemical constituents and a 90-day rat inhalation study. Based on the findings of these studies, the authors concluded that the addition of the combined ingredients, including β-Ionone at levels up to 6 ppm, “did not increase the overall toxicity of cigarette smoke”.

Renne et al., (2006) evaluated the effects of tobacco flavouring and casing ingredients on both mutagenicity, and a number of physiological parameters in Sprague-Dawley (SD) rats. Test cigarettes containing a mixture of either 165 low-uses or eight high-use flavouring ingredients which included ionone, beta at 0.13ppm, were compared to a typical commercial tobacco blend without flavouring ingredients. The Ames assay (TA 98, 100,102, 1535 and 1537 ±S9) did not show any increase in Mutagenicity from “low” or “high” cigarette smoke condensate compared to the control. SD rats were exposed by nose-only inhalation for 1h/day, 5 days/wk for 13 weeks to smoke at concentrations of 0.06, 0.2 or 0.8mg/L from the test or reference cigarettes, or to air only. Plasma nicotine, COHb and respiratory parameters were measured periodically. Rats were necropsied after 13wk of exposure or following 13 wk of recovery from smoke exposure. Biological endpoints assessed included; clinical appearance, body weight, organ weights, and lesions (both gross and microscopic). The results of these studies did not indicate any consistent differences in toxicological effects between smoke from cigarettes containing the flavouring or casing ingredients and reference cigarettes.

A recent study investigated the effect of cigarettes, containing various additives in three combinations, in a 90 day nose-only smoke inhalation study in rats [Vanscheeuwijck et al., 2002]. These ingredients included β-Ionone at 6ppm, a level described as a multiple of its typical use in a US cigarette. The data from this study along with that from a number of other biological and chemical studies indicate that the addition of the combined ingredients “did not increase the inhalation toxicity of the smoke, even at the exaggerated levels used” [Vanscheeuwijck et al., 2002].

Roemer (2014) and Schramke (2014) reported on a testing program designed to evaluate the potential effects of 350 ingredients added to an experimental kretek cigarette on selected biological and chemical endpoints. The studies performed included a bacterial mutagenicity screen [Ames assay] a mammalian cell cytotoxicity assay

[neutral red uptake], Mouse Lymphoma assay, determination of smoke chemical constituents, a 4-day in vivo micronucleus assay and a 90-day rat inhalation study. Based on the results of these studies, the authors concluded that the addition of ingredients commonly used in the manufacture of kretek cigarettes, including Beta Ionone at levels up to 3 ppm, did not change the overall in vivo/vitro toxicity profile of the mainstream smoke.

Roemer et al., [2002], reported on a study in which cigarettes containing various additives in three different combinations were produced. Smoke condensates prepared from these cigarettes were then tested in two different in vitro assays. The mutagenicity of the smoke condensate was assayed in the Salmonella plate incorporation [Ames] assay with tester strains TA98, TA100, TA102, TA1535 and TA1537 in the presence and absence of an S9 metabolic activation system. The cytotoxicity of the gas/vapour phase and the particulate phase was determined in the neutral red uptake assay with mouse embryo BALB/c 3T3 cells.The authors concluded that the in vitro mutagenicity and cytotoxicity of the cigarette smoke was not increased by the addition of the ingredients which included β-Ionone at levels up to 6ppm [a multiple of its typical use in a US cigarette] [Roemer et al., 2002].

Baker et al., [2004]; examined the effects of the addition of 482 tobacco ingredients upon the biological activity and chemistry of mainstream smoke. The ingredients, essentially different groups of flavourings and casings, were added in different combinations to reference cigarettes. The addition of ß ionone at 12 ppm was determined not to have affected the mutagenicity of the total particulate matter (TPM) of the smoke in either the Ames, in vitro micronucleus assay or the neutral red assay when compared with that of the control cigarettes [Baker et al., 2004].

Roemer (2014) and Schramke (2014) reported on a testing program designed to evaluate the potential effects of 350 ingredients added to an experimental kretek cigarette on selected biological and chemical endpoints. The studies performed included a bacterial mutagenicity screen [Ames assay] a mammalian cell cytotoxicity assay [neutral red uptake], Mouse Lymphoma assay, determination of smoke chemical constituents, a 4-day in vivo micronucleus assay and a 90-day rat inhalation study. Based on the results of these studies, the authors concluded that the addition of ingredients commonly used in the manufacture of kretek cigarettes, including Beta Ionone at levels up to 3 ppm, did not change the overall in vivo/vitro toxicity profile of the mainstream smoke.

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