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TOXICOLOGICAL EVALUATIONS

BG RCI

Kurfürsten-Anlage 62 · D-69115 Heidelberg, Germany
Telefon: +49 6221 5108-28451
E-Mail: toxikologischebewertungen@bgrci.de
Internet: www.bgrci.de/toxicologicalevaluations

TOXICOLOGICAL EVALUATION

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Isononanoic acid No. 276

CAS No. 26896-18-4

BG Chemie
Berufsgenossenschaft der chemischen Industrie

BG Chemie
P.O.B. 10 14 80, 69004 Heidelberg, Germany
Telephone: +49 (0) 6221 523 400
E-Mail: ToxikologischeBewertungen@bgchemie.de
Internet: www.bgchemie.de/toxicologicalevaluations

Isononanoic acid

The term, isononanoic acid, which actually refers to 7-methyloctanoic acid (CAS No. 26896-18-4), is used for a technical-grade product consisting of a mixture of isomeric branched-chain carboxylic acids with 9 carbon atoms. Depending on the production process employed, the mixture contains more than 90% 3,5,5-trimethylhexanoic acid (CAS No. 3302-10-1) or 2,2,4,4-tetramethylpentanoic acid (CAS No. 3302-12-3) as its main component. The present Toxicological Evaluation discusses data on both pure 7-methyloctanoic acid and technical-grade isononanoic acid.

1 Summary and assessment

In the rat, the LD_{50} values found for oral administration of technical-grade isononanoic acid indicate that the product is of low toxicity or not harmful; the LD_{50} value for technical isononanoic acid consisting of approx. 90% 3,5,5-trimethylhexanoic acid and 10% other trimethylhexanoic acids, including 3,4,5-trimethylhexanoic acid, is 3135 mg/kg body weight, and an LD_{50} value of 1160 mg/kg body weight has been reported for technical-grade isononanoic acid described only as a mixture of isomers of 99.7% purity containing between 0.25% and 0.3% main contaminants. Following administration of lethal doses, animals died within 24 to 48 hours, exhibiting unspecific signs of toxicity (including disturbances of balance, lying on the abdomen, sedation, ataxia, diarrhoea and difficulty in breathing). Surviving animals were free of signs of toxicity 48 hours after administration. Post-mortems on animals which died intercurrently and on those which were sacrificed at the end of the study revealed no macroscopically discernible changes and only unspecific findings, respectively. The LD_{50} value for dermal exposure of rats is reported as > 2000 mg/kg body weight, on the basis of which isononanoic acid is also of low toxicity when administered via this route. An Alarie test to determine the sensory irritation threshold yielded an RD_{50} value (concentration at which mean respiratory frequency is reduced by 50%) of 420 mg/m³. In this test, rats survived 3-hour exposure to levels of 200 to 800 mg/m³ and a subsequent one-week observation period.

A subacute oral study conducted in the Wistar rat in accordance with OECD guideline No. 407 and Directive 96/54/EEC at levels of up to 200 mg/kg body weight/day has demonstrated the liver and kidneys to be the target organs of systemic toxicity of isononanoic acid (94.9% 3,5,5-trimethylhexanoic acid). Histopathology revealed liver changes in the form of minimal to moderate fatty infiltration of the peripheral liver cells occurring in a dose-related manner at dose levels of and above 50 mg/kg body weight, especially in females. Organ weight increases and alterations of hepatic function (increased alanine aminotransferase activity, in females only) were noted only in the highest dose group, which was treated at 200 mg/kg body weight. A peroxisome-proliferative effect of isononanoic acid was suggested by the increase in cyanide-insensitive palmitoyl-CoA oxidation in total liver homogenates from males treated at and above 50 mg/kg body weight and females treated at 200 mg/kg body weight. Kidney findings included $\alpha_{2\mu}$-globulin nephropathy, which is specific to the male rat. At and above 10 mg/kg body weight, the lowest dose tested, diffuse $\alpha_{2\mu}$-globulin accumulation occurred in the epithelia and in some instances also in the proximal tubular lumen in males. From 50 mg/kg body weight, males exhibited multifocal tubular dilation in association with cellular deposits in the lumen and increased relative kidney weight. The males' urine sediments contained degenerated transitional epithelial cells from 10 mg/kg body weight and renal tubular epithelial cells, squamous epithelial cells, granular casts and epithelial cell casts at and above 50 mg/kg body weight. In males, urinary volume was increased while specific gravity was decreased from 50 mg/kg body weight, the urine containing blood. Females only showed increased kidney weights from 50 mg/kg body weight and increased urinary specific gravity at 200 mg/kg body weight. Liver changes were completely reversible, kidney changes partially reversible during a recovery period of 14 days. The comprehensive "Functional Observational Battery" testing and motor activity assessments carried out as part of the study and the histopathological examination of the peripheral and central nervous system gave no indication of any specific neurotoxicity for isononanoic acid. All other organs and tissues examined in accordance with the above-mentioned guidelines were also histopathologically normal in animals treated at 200 mg/kg body weight/day. The *no observed adverse effect level* (NOAEL) for female rats was 10 mg/kg body weight. As male rats exhibited diffuse $\alpha_{2\mu}$-globulin accumulation in the renal tubules and degenerated transitional epithelial cells in their urine sediments even at the

lowest test dose, 10 mg/kg body weight, the NOAEL for the male rat is less than 10 mg/kg body weight. However, when assessing the subacute toxicological potential of isononanoic acid it is necessary to consider that $\alpha_{2\mu}$-globulin nephropathy constitutes a feature specific to the male rat and occurs in no other mammalian species, including humans. With this species- and sex-specific effect taken into account, the NOAEL for male rats is also 10 mg/kg body weight.

According to less recent skin and mucous membrane irritancy studies, repeated daily dermal application of technical-grade isononanoic acid (approx. 90% 3,5,5-trimethylhexanoic acid and 10% other trimethylhexanoic acids, including 3,4,5-trimethylhexanoic acid) to the skin of rabbits for 5 days results in necrotic changes. Similar treatment with a 10-percent and a 5-percent formulation is tolerated without findings. Single intracutaneous administration to rabbits of 10-percent and 5-percent formulations of the product have been found to cause necrosis at the injection sites. The skin damage seen upon repeated administration of the undiluted product was assessed by the investigators as considerable. Single instillation of the undiluted product into the conjunctival sac of the rabbit eye has been reported to cause mild reddening and glassy swelling of the conjunctiva and slight clouding of the cornea. At 72 hours after instillation, these findings had not yet cleared up completely. Instillation of 1-percent, 5-percent and 10-percent formulations of the test sample led to very mild to mild concentration-dependent reddening of the conjunctivae, which persisted for 7 or 48 hours, depending on concentration. Treatment with an 0.1-percent formulation was tolerated without findings. Based on these results, the investigators recommended that skin and mucous membrane contact with the product be avoided. Single application of technical-grade isononanoic acid (mainly 3,5,5-trimethylhexanoic acid; no further details) to the skin or eyes of rabbits resulted in mild reversible irritation in studies carried out in accordance with Directive 84/449/EEC. Based on the classification criteria set forth in Directive 83/467/EEC, the test substance was assessed as not irritating to the skin and eye. A different technical isononanoic acid product (a mixture of isomers of 99.7% purity containing between 0.25% and 0.3% main contaminants; no further details) caused severe irritation to the rabbit skin and the rabbit eye in studies carried out in accordance with OECD guidelines Nos. 404 and 405 and was evaluated as severely irritating to the skin and moderately irritating to the eye.

A one-generation feeding study of isononanoic acid in Crl:CD®BR-VAF/Plus® rats which was conducted in accordance with Directives 67/548/EEC and 88/302/EEC gave no indication of any specific reproductive toxicity potential from dietary administration of the chemical at levels of up to 0.5%. No treatment-related effects were noted on sperm count, motility or morphology or on mating behaviour, mating efficiency, gestation or parturition. Macroscopic examination and parental reproductive organ weight determinations also gave no remarkable findings. Adverse effects in the F_1 animals, such as body weight decreases in the two highest dose groups and reduced live birth index, reduced survival indices and delayed postnatal development in the top dose group, were restricted to those dose groups which also exhibited parental toxicity (decreased body weights, increased liver weights). External examination and necropsy of F_1 animals treated with isononanoic acid revealed no treatment-related abnormalities. The *no observed adverse effect level* (NOAEL) for dietary isononanoic acid (approx. 66 and 81 mg/kg body weight/day based on parental food consumption recorded for males and females, respectively, shortly before mating) was 0.12% for both parental animals and F_1 offspring.

Studies carried out in accordance with OECD guidelines Nos. 471 and 472 as well as Directive 79/831/EEC in *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538 as well as *Escherichia coli* WP2uvrA have given no indications of any genotoxic effects from technical-grade isononanoic acid (3,5,5-trimethylhexanoic acid; purity not specified), either with or without metabolic activation.

There is no evidence from human experience to indicate that isononanoic acid has a sensitising potential.

The German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area (“MAK-Kommission”) has listed isononanoic acid in the “Yellow Pages” (“Substances being Examined for the Establishment of MAK Values and BAT Values”) of the List of MAK and BAT Values on the suggestion of BG Chemie in order that a MAK value be established for the chemical.

2 Name of substance

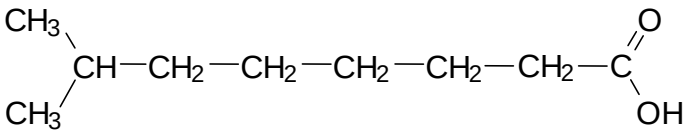
2.1	Usual name	Isononanoic acid
2.2	IUPAC name	Isononanoic acid
2.3	CAS No.	26896-18-4 (7-methyloctanoic acid) 3302-10-1 (3,5,5-trimethylhexanoic acid) 3302-12-3 (2,2,4,4-tetramethylpentanoic acid
2.4	EINECS No.	248-092-3 (7-methyloctanoic acid) 221-975-0 (3,5,5-trimethylhexanoic acid)

3 Synonyms, common and trade names

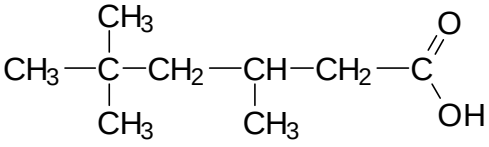
CeKanoic9
CeKanoic C9 acid
INA
ISO CK 9
Isononansäure
Isononylic acid
Isopelargonic acid
i-Nonansäure

4 Structural and molecular formulae

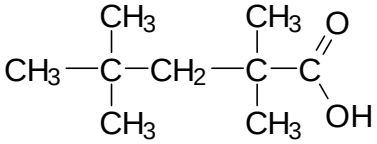
4.1 Structural formula



7-Methyloctanoic acid
(CAS No. 26896-18-4)



3,5,5-Trimethylhexanoic acid
(CAS No. 3302-10-1)



2,2,4,4-Tetramethylpentanoic acid
(CAS No. 3302-12-3)

4.2 Molecular formula $C_9H_{18}O_2$

5 Physical and chemical properties

5.1 Molecular mass, g/mol 158.24

5.2 Melting point, °C ca. –70 (purity not specified, and technical grade isononanoic acid containing ≥ 97.5% 3,5,5-trimethylhexanoic acid, depending on the source of information)
(Hoechst, 1991; Hüls, 1995; Riemenschneider, 1986)
< –65 (technical-grade isononanoic acid containing approx. 97.5% 3,5,5-trimethylhexanoic acid) (ExxonMobil, 2001)
< –65 (technical-grade isononanoic acid containing 100% 3,5,5-trimethylhexanoic acid)
(Deutsche Exxon, 1998; Exxon, 1998)
< –60 (technical-grade isononanoic acid containing primarily or approx. 90% 3,5,5-trimethylhexanoic acid, depending on the source of information)
(Hoechst, 1985 a, b, 1988; Hüls, 1994; Riemenschneider, 1986; Celanese, 1999)

5.3	Boiling point, °C	228–241 (technical-grade isononanoic acid containing approx. 97.5 or 100% 3,5,5-trimethylhexanoic acid, depending on the source of information) (ExxonMobil, 2001; Deutsche Exxon, 1998) 230–240 (at 1013 hPa, technical-grade isononanoic acid containing ≥ 97.5% 3,5,5-trimethylhexanoic acid) (Hüls, 1995) 232–236 (technical-grade isononanoic acid containing primarily 3,5,5-trimethyl-hexanoic acid; no further details) (Falbe and Regitz, 1997) 233–245 (technical-grade isononanoic acid containing approx. 90% 3,5,5-tri-methylhexanoic acid) (Riemenschneider, 1986) 235 (technical-grade isononanoic acid containing primarily 3,5,5-trimethyl-hexanoic acid; no further details) (Hoechst, 1985 a, b; Celanese, 1999) 240–255 (purity not specified) (Hüls, 1994)
5.4	Vapour pressure, hPa	< 0.01 (at 20 °C; purity not specified) (Hüls, 1994) 0.04 (at 20 °C) and 2.6 (at 130 °C; tech-nical-grade isononanoic acid containing ≥ 97.5% 3,5,5-trimethylhexanoic acid) (Hüls, 1995) < 0.1 (at 20 °C; purity not specified) (Hoechst, 1991) < 0.1 (at 20 °C) and approx. 0.2 (at 50 °C; technical-grade isononanoic acid consisting of 3,5,5-trimethylhexanoic acid; purity not specified) (Hoechst, 1988) 0.63 (at 50 °C) and 10.88 (at 100 °C; 100% 3,5,5-trimethylhexanoic acid) (Deutsche Exxon, 1998)

5.5	Density, g/cm³	0.895–0.902 (technical-grade isononanoic acid containing primarily 3,5,5-trimethylhexanoic acid, and purity not specified, depending on the source of information) (Falbe and Regitz, 1997; Riemenschneider, 1986) 0.897–0.901 (technical-grade isononanoic acid containing approx. 90% 3,5,5 trimethylhexanoic acid) (Riemenschneider, 1986) 0.898 (technical-grade isononanoic acid containing ≥ 97.5% 3,5,5-trimethylhexanoic acid) (Hüls, 1995) 0.899 (technical-grade isononanoic acid containing approx. 97.5 or 100% 3,5,5-trimethylhexanoic acid, depending on the source of information) (ExxonMobil, 2001; Deutsche Exxon, 1998; Hoechst, 1985 a, b; Celanese, 1999) 0.9 (technical-grade isononanoic acid containing 3,5,5-trimethylhexanoic acid, and purity not specified, depending on the source of information) (Hoechst, 1988; Hüls, 1994)
5.6	Solubility in water	Largely insoluble, 0.3% v/v at 20 °C, absorbs 1.3–1.6% water at 20 °C (purity not specified) (Riemenschneider, 1986) 0.3% (technical-grade isononanoic acid containing primarily 3,5,5-trimethylhexanoic acid; no further details) (Hoechst, 1985 a, b) ca. 0.2% (technical-grade isononanoic acid containing ≥ 97.5% 3,5,5-trimethylhexanoic acid, and purity not specified, depending on the source of information) (Hüls, 1994, 1995) 3 g/l (at 20 °C, mixture of isomeric isononanoic acids, primarily 3,5,5-trimethylhexanoic acid) (Celanese, 1999) 730 mg/l and 0.08% (at 25 °C, 100% 3,5,5-trimethylhexanoic acid) (Deutsche Exxon, 1998)

5.7	Solubility in organic solvents	Miscible with most organic solvents (Hüls, 1995; Riemenschneider, 2002)
5.8	Solubility in fat	Partition coefficient log P_{ow}: ca. 3.1 (purity not specified) (Hoechst, 1991) Partition coefficient log P_{ow}: ca. 3.3 (calculated; purity not specified) (EC, 2000)
5.9	pH value	3.5 (technical-grade isononanoic acid containing 3,5,5-trimethylhexanoic acid, and purity not specified, depending on the source of information) (Celanese, 1999; Hoechst, 1988; Hüls, 1994)
5.10	Conversion factor	1 ml/m³ (ppm) ≙ 6.47 mg/m³ 1 mg/m³ ≙ 0.154 ml/m³ (ppm) (at 1013 hPa and 25 °C)

6 Uses

In alkyd resins, drying agents for varnishes and oil paints, lubricating oil additives, stabilisers and plasticisers for PVC polymers, fungicides and basic materials for cosmetics (Falbe and Regitz, 1997; Hüls, 1995; Riemenschneider, 2002). Intermediate in the production of metal salts, acid chlorides, esters and other chemicals for numerous applications (3,5,5-trimethylhexanoic acid; Deutsche Exxon, 1998).

7 Experimental results

7.1 Toxicokinetics and metabolism

No information available.

7.2 Acute and subacute toxicity

Acute toxicity

For technical-grade isononanoic acid (approx. 90% 3,5,5-trimethylhexanoic acid and 10% other trimethylhexanoic acids, including 3,4,5-trimethyl-

hexanoic acid), an oral LD_{50} of 3135 mg/kg body weight was ascertained in the rat. A sesame-oil solution of the product was administered to female SPF-Wistar-K rats by oral gavage. The animals were placed under observation for 14 days. Lethally intoxicated animals exhibited disturbances of balance and died within 1.5 to 48 hours, lying on the abdomen in a narcosis-like state (with hardly any reflexes). Necropsy of the deceased animals revealed no remarkable macroscopic findings (Hoechst, 1970).

For another technical-grade isononanoic acid product (mixture of isomers 99.7% pure, main contaminants 0.25 to 0.3%; no further details), an LD_{50} of 1160 mg/kg body weight was ascertained following oral administration to rats. The study was carried out in accordance with OECD guideline No. 401. The undiluted test substance was administered to dose groups of 10 male and 10 female rats of the Bor:WISW (SPF TNO) strain by oral gavage. The animals showed treatment-related signs of toxicity starting 15 to 30 minutes after administration. These included ruffled fur, squatting posture, reeling, mild sedation and ataxia in some instances and, at times, lying on the abdomen. Later, further signs were noted, including lacrimation, slowed movements, diarrhoea, breathing difficulties, moderate to severe sedation and ataxia, gasping vocalisations, hypothermia, tremor and at times widely opened eyes. Lethally intoxicated animals died 24 to 31 hours after administration. Necropsy revealed excessively full urinary bladder, cellular texture of the liver and kidney surfaces as well as hyperaemia of the gastric and intestinal mucosa, urinary bladder mucosa, lungs, pancreas and subcutaneous fatty tissue. The survivors were free of clinical signs of toxicity 48 hours after administration. Terminal necropsy at the end of the 14-day observation period revealed partial, in some instances severe, hyperaemia of the intestinal mucosal tissues in some animals (Hüls, 1986 a).

A dermal LD_{50} value for male and female rats was determined as > 2000 mg/kg body weight (no further details; RCC, 1988).

A synthetic metalworking fluid (the cutting oil MWF “A”) and its major components were evaluated using an Alarie test to determine the threshold of sensory irritation in groups of 4 male Swiss-Webster mice (weighing 24 to 28 g) per concentration. The cutting oil consisted of 1 to 10% isononanoic acid (isomeric composition not specified), 10 other components and approx. 60% water. A whole-body plethysmograph was used to measure pressure changes created during inhalation and exhalation as well as res-

piratory frequency during 180-minute exposure to isononanoic acid at concentration levels of approx. 200 to 800 mg/m³ (analytical concentrations of 172 to 755 mg/m³). The animals were then exposed to air alone for 20 minutes and subsequently kept under observation for one week. There were concentration-dependent decreases in respiratory frequency by approx. 20, 40, 55 and 55% at approx. 200, 300, 400 and 800 mg/m³, respectively. Maximum decrease in respiratory frequency was observed during the third hour of exposure. The RD_{50} value (concentration at which mean respiratory frequency was reduced by 50%) for isononanoic acid was 420 mg/m³ (95% confidence interval 337 to 589 mg/m³). Isononanoic acid evoked predominantly pulmonary irritation, which persisted throughout the entire exposure and reached a plateau in response within the first hour. The chemical also evoked sensory irritation during the first hour. Tidal volume was not affected in a significant manner by the metalworking fluid or its individual components. The respiratory frequency did not return to normal levels during the 20-minute recovery phase immediately after the 3-hour exposure. No mortality was observed during the 1-week observation period (Detwiler-Okabayashi and Schaper, 1996).

Subacute toxicity

The subacute toxicity of isononanoic acid was investigated in a study conducted in male and female Wistar rats (CrlGlxBrlHan:WI strain) in accordance with OECD guideline No. 407 and Directive 96/54/EEC. Groups of 5 or 10 animals/sex and concentration received daily treatment with isononanoic acid (94.9% 3,5,5-trimethylhexanoic acid) in bidistilled water containing 0.1% Cremophor for 28 days at dose levels of 0 (control), 10, 50 or 200 mg/kg body weight. Groups of 5 males and 5 females treated at 0 (control) or 200 mg/kg body weight were kept under observation as recovery groups for 14 days after the end of the treatment period. The scope of investigation as set forth in OECD guideline No. 407 and Directive 96/54/EEC was expanded to include neurotoxicity evaluations for animals from all dose groups by means of comprehensive “Functional Observational Battery” testing and motor activity assessments. The findings obtained for the groups treated with isononanoic acid are summarised in Table 1 below.

Beginning of Table 1

Table 1. Subacute toxicity of isononanoic acid in the Wistar rat: Findings after 4-week administration by gavage and a 2-week recovery period (BASF, 2002)				
	10 mg/kg body weight	50 mg/kg body weight	200 mg/kg body weight	200 mg/kg body weight, recovery group
No. of animals	5 M, 5 F	5 M, 5 F	5 M, 5 F	5 M, 5 F
Clinical examination				
Mortality, body weight data, food efficiency, clinical signs	no effects	no effects	no effects	no effects
Food consumption	no effects	no effects	decreased (F, first study week)	no effects
FOB, motor activity	no effects	no effects	FOB: no findings; motor activity decreased (F)	no effects
Clinical chemistry and haematology parameters				
Haematology, including clotting analyses	no effects	no effects	no effects	no effects
Alanine aminotransferase activity	no effects	no effects	increased (F)	no effects
Serum protein levels	no effects	no effects	reduced albumin (M), reduced total protein and globulin (F)	reduced globulin (F)
Cyanide-insensitive palmitoyl-CoA oxidation in the liver	no effects	increased (F)	increased (M, F)	no effects
Other clinical chemistry parameters determined according to the guidelines mentioned above	no effects	no effects	no effects	no effects
Urinalysis				
Urinary volume	no effects	increased (M)	increased (M, F)	no effects
Specific gravity	no effects	decreased (M)	decreased (M, F)	no effects
Urinary blood	no effects	present (M)	present (M)	no effects
Turbidity of the urine	no effects	no effects	present (M)	no effects
Increased number of cells in urine sediment as revealed by microscopy	degenerated transitional epithelial cells (M)	renal tubular epithelial cells, degenerated transitional epithelial cells, squamous epithelial cells, granular casts and epithelial cell casts (M)	renal tubular epithelial cells, degenerated transitional epithelial cells, squamous epithelial cells, granular casts and epithelial cell casts (M)	no effects
Other urinalysis parameters determined according to the guidelines mentioned above	no effects	no effects	no effects	no effects

Table 1. Subacute toxicity of isononanoic acid in the Wistar rat: Findings after 4-week administration by gavage and a 2-week recovery period (BASF, 2002)

		10 mg/kg body weight	50 mg/kg body weight	200 mg/kg body weight	200 mg/kg body weight, recovery group
Macroscopic/histopathological examination					
Liver	Absolute organ weight	no effects	no effects	+ 19% (M)	no effects
	Relative organ weight	no effects	no effects	+ 17% (M) + 22% (F)	no effects
	Macroscopic examination	no effects	no effects	no effects	no effects
	Histopathological examination	no effects	peripheral fatty infiltration of liver cells (minimal in 4/5 F)	peripheral fatty infiltration of liver cells (mild to moderate in 5/5 F, mild in 1/5 M)	no effects
Kidney	Absolute organ weight	no effects	no effects	+ 26% (M)	+ 10% (M)
	Relative organ weight	no effects	+ 11% (M) + 9% (F)	+ 24% (M) + 12% (F)	+ 15% (M)
	Macroscopic examination	no effects	no effects	no effects	no effects
	Histopathological examination	diffuse accumulation of $\alpha_{2\mu}$-globulin in the epithelia and in some instances also in the proximal tubular lumen (4/5 M)	diffuse accumulation of $\alpha_{2\mu}$-globulin in the epithelia and in some instances also in the proximal tubular lumen (M); multifocal tubular dilation with cellular detritus deposited in the lumen (1/5 M)	diffuse accumulation of $\alpha_{2\mu}$-globulin in the epithelia and in some instances also in the proximal tubular lumen (M); multifocal tubular dilation with cellular detritus deposited in the lumen (2/5 M)	diffuse accumulation of $\alpha_{2\mu}$-globulin in the epithelia of the proximal tubules (M), multifocal tubular dilation (2/3 M), regenerative proliferation (basophilic tubules, 4/5 M)
Other macroscopic and histopathological examinations					
Other organs and tissues[1] examined according to the guidelines mentioned above		not examined	not examined	no effects	not examined

FOB Functional Observational Battery

[1] brain, thyroid gland, thymus, trachea, heart, spleen, adrenal glands, ovaries, uterus, prostate gland, stomach, duodenum, ileum, jejunum, caecum, colon, rectum, mandibular and mesenteric lymph nodes, sciatic nerve, spinal cord, bone marrow

End of Table 1

Subacute oral administration of isononanoic acid at dose levels of up to 200 mg/kg body weight had no effect on mortality, body weight data or water consumption in treated animals. Food consumption was significantly reduced only in the top-dose females and only during the first week of the study. Food efficiency remained unchanged. Haematology (including clotting analyses) and the usual clinical chemistry parameters were unremarkable, apart from increased alanine aminotransferase activity and de-

creased total protein and globulin levels in females and a decreased albumin level in top-dose males. The kidneys and the liver proved to be the target organs for the systemic toxicity of isononanoic acid. Liver changes occurred in the form of fatty infiltration of the peripheral liver cells in a dose-related manner in 4 out of 5 females in the intermediate and 5 out of 5 females in the top dose group, but only in 1 out of 5 males in the top dose group. Increase in liver weight and, in females, indications of altered hepatic function (increased alanine aminotransferase activity) were noted only in the top dose group. A peroxisome-proliferative effect of isononanoic acid was suggested by an increase in cyanide-insensitive oxidation in whole liver homogenate observed in intermediate and top-dose males and in top-dose females. Males developed $\alpha_{2\mu}$-globulin nephropathy in all dose groups. At and above 10 mg/kg body weight, the lowest dose tested, diffuse $\alpha_{2\mu}$-globulin accumulation occurred in the epithelia and in some instances also in the proximal tubular lumen. From 50 mg/kg body weight, males exhibited multifocal tubular dilation in association with cellular deposits in the lumen and increased relative kidney weight. The males' urine sediments contained degenerated transitional epithelial cells from the lowest dose and renal tubular epithelial cells, squamous epithelial cells, granular casts and epithelial cell casts at the intermediate and higher doses. Furthermore, urinary volume was increased while specific gravity was decreased at and above that dose, and the urine contained blood. Urine samples from males treated at 200 mg/kg body weight were cloudy. Females only showed increased kidney weights from the intermediate dose and increased urinary specific gravity in the top dose group. There were no remarkable findings in the females with respect to kidney histopathology or urine sediments. Liver changes were completely reversible and kidney changes partially reversible during the 14-day observation period. The study gave no indication of any neurotoxicity for isononanoic acid; the comprehensive FOB test results were unremarkable and the peripheral and central nervous system showed no abnormal histopathological findings. The reduced motor activity noted in females treated at 200 mg/kg body weight was evaluated by the investigators as constituting a secondary consequence of general systemic toxicity at this level rather than a specifically neurotoxic effect. A *no observed adverse effect level* (NOAEL) was not achieved for males given the lowest dose tested, 10 mg/kg body weight, as these males exhibited diffuse $\alpha_{2\mu}$-globulin accumulation in the epithelia of the proximal renal tubules and their urine sediment contained degenerated

transitional epithelial cells. However, $\alpha_{2\mu}$-globulin accumulation is a species-specific finding which occurs only in the male rat and has no relevance to human risk assessment. With this species- and sex-specific effect taken into account, the NOAEL for male and female rats determined in this study was 10 mg/kg body weight (see also Section 7.10; BASF, 2002).

The dose levels tested in the subacute study discussed above (BASF, 2002) were selected on the basis of the results of a 14-day range-finding study in which groups of 3 Wistar rats/sex and dose were treated by oral gavage at levels of 0 (control), 300 or 1000 mg/kg body weight/day. In the top dose group, 1 out of 6 animals died intercurrently and findings included clinical signs indicative of reduced general condition (e.g. laboured breathing, salivation, altered gait, lateral position, urine-stained coat), increased water consumption, decreases in body weight and body weight gain, and increased liver weights. Macroscopic kidney and liver changes were noted from 100 mg/kg body weight and 300 mg/kg body weight, respectively (discoloured kidneys, livers with prominent acinar patterns; BASF, 2001, 2002).

7.3 Skin and mucous membrane effects

The skin irritancy of technical-grade isononanoic acid (mainly 3,5,5-trimethylhexanoic acid; no further details) was investigated in the albino New Zealand rabbit in a study conducted in accordance with Directive 84/449/EEC, which in substance fulfils the requirements set forth in OECD guideline No. 404. Following 4-hour semi-occlusive application of 0.5 ml isononanoic acid to the intact depilated dorsal skin, 3 of the exposed animals displayed large areas with light-brown discoloration and clearly circumscribed erythema which persisted for 7 days, or mild erythema, while another animal showed very mild erythema which persisted for 72 hours. From day 7 after application until the end of the 14-day observation period, the skin of all 3 animals was scaly, dry and chapped. The surface of the skin peeled off in fine scales without scar formation. Based on the scores for the individual findings for erythema and eschar formation, and oedema formation at the assessments carried out 24, 48 and 72 hours after application, the following mean values were calculated: erythema and eschar formation 1, and oedema formation 0. On the basis of the classification criteria set forth in EC Directive 83/467/EEC and the results of this trial, isononanoic acid was evaluated as not irritating to the skin (Hoechst, 1985 a).

In a skin irritation test carried out in accordance with OECD guideline No. 404, another isononanoic acid product (mixture of isomers, 99.7% pure, main contaminants 0.25 to 0.3%; no further details) was evaluated as severely irritating to the skin. Following 4-hour occlusive exposure of the intact depilated dorsal skin of 3 rabbits (males, small white Himalayan, Chbb-SPF strain) to the undiluted test substance, moderate to severe erythema and mild to moderate oedema were observed up to 72 hours after application. Subsequently, large scabs formed, which had fallen off by the end of the 14-day observation period, or were in the process of detachment at that time. An irritation index of 5.67 out of a maximum irritation index of 8 was determined. In addition, on the basis of Directive 79/831/EEC, mean values of 3.00 were calculated for both erythema and oedema formation (Hüls, 1986 b).

Over a period of 5 days, technical-grade isononanoic acid (approx. 90% 3,5,5-trimethylhexanoic acid and 10% other trimethylhexanoic acids, including 3,4,5-trimethylhexanoic acid) was administered daily to groups of 5 rabbits ("Gelb-Silber") as 0.5 ml applications of the undiluted acid or a 10- or 5-percent formulation of the chemical to the intact depilated skin of the flanks. Sesame oil served as the solvent. Application of the undiluted product caused reddening of the flank skin in all rabbits after the first or second treatment. At the end of the study (after the 5th application), there was necrosis of the flank skin in all rabbits. The 10- and 5-percent formulations were tolerated without any reactions by all rabbits. The investigators evaluated the skin damage caused by application of the undiluted product as being considerable and recommended that skin contact with the product be avoided (Hoechst, 1970).

Groups of 5 rabbits ("Gelb-Silber") were treated with 0.02 ml doses of formulations containing 10, 5, 1, 0.1, 0.01 or 0.001% technical-grade isononanoic acid (approx. 90% 3,5,5-trimethylhexanoic acid and 10% other trimethylhexanoic acids, including 3,4,5-trimethylhexanoic acid) by intracutaneous injection into the depilated skin of the flank (Barail test). The controls were treated with the vehicle, sesame oil. In all rabbits, the 10- and 5-percent formulations led to a necrotic centre surrounded by a red halo at the injection site. Injections of the other formulations were tolerated without any reactions (Hoechst, 1970).

Furthermore, the mucous membrane effects of technical-grade isononanoic acid (mainly 3,5,5-trimethylhexanoic acid; no further details) were investi-

gated in the albino New Zealand rabbit in a study conducted in accordance with EC Directive 84/449/EEC, which in substance fulfils the requirements set forth in OECD guideline No. 405. A 0.1 ml dose of the undiluted chemical was instilled into one conjunctival sac in each of 3 animals. The treated eyes were thoroughly washed with physiological saline solution at all time points of assessment. Up to 72 hours after instillation, the animals' conjunctivae showed reddening which ranged from marked hyperaemia of the blood vessels to a diffuse, intense red colour. The conjunctivae were slightly to clearly swollen, and the iris was reddened. At 60 minutes after instillation, the cornea exhibited scattered to diffuse areas of opacity. The signs of irritation were accompanied by clear ocular discharge. After 7 days, one animal was found to have slight hair loss around the treated eye. At that time point, all other signs of irritation were reversible. Based on the scores for the individual findings at 24, 48 and 72 hours, the following mean scores were observed: clouding of the cornea 0, reddening of the conjunctiva 2.1, iritis 0.8 and conjunctival swelling 1.7. On the basis of the classification criteria set forth in EC Directive 83/467/EEC and the results of this trial, isononanoic acid was evaluated as not irritating to the eye (Hoechst, 1985 b).

In a mucous membrane irritation test carried out in accordance with OECD guideline No. 405, another isononanoic acid product (mixture of isomers, 99.7% pure, main contaminants 0.25 to 0.3%; no further details) was evaluated as moderately irritating to the eye. At 72 hours after instillation of the undiluted test substance into the eye or palpebral fissure of 3 rabbits (males, small white Himalayan, Chbb-SPF strain), the eyes were assessed with sodium fluorescein solution and subsequently rinsed out with physiological saline solution. Application of the test substance led to translucent corneal opacity, reddening of the iris with pericorneal injection and haemorrhagic spots on the iris as well as a diffuse crimson to beefy redness of the conjunctivae accompanied by slight swelling and exudation. The findings cleared up as of day 17 after instillation. In the 2 other animals, there were findings even at the end of a 21-day observation period (punctiform scattered or diffuse areas of opacity on less than 25% of the area of the cornea and pericorneal injection of the iris or translucent cloudiness of less than 25% of the area of the cornea). The irritation index was calculated as 41.84 out of a maximum irritation index of 110. In addition, on the basis of EC Directive 79/831/EEC, the mean values of the scores for the effects on the

cornea, iris and conjunctiva seen at 24, 48 and 72 hours were calculated to be 1.78 for corneal opacity, 1.00 for the iris findings, 3.00 for reddening of the conjunctiva and 1.00 for conjunctival swelling (Hüls, 1986 c).

The mucous membrane effects of technical-grade isononanoic acid (approx. 90% 3,5,5-trimethylhexanoic acid and 10% other trimethylhexanoic acids, including 3,4,5-trimethylhexanoic acid) was investigated in groups of 5 rabbits (”Gelb-Silber”) by a single 0.1 ml instillation of the undiluted acid or a 10-, 5-, 1- or 0.1-percent formulation of the chemical into the conjunctival sac. Sesame oil served as the solvent. Assessments were carried out 1, 3, 7, 24, 48, 72 and 144 hours following instillation. The undiluted product resulted in mild general reddening and slightly glassy swelling of the conjunctiva in all 5 animals. In 3 out of 5 rabbits the cornea was slightly cloudy. At 72 hours after instillation, the findings had not yet cleared up completely. In all animals, instillation of the 1-, 5-and 10-percent formulations led to very mild to mild concentration-dependent reddening of the conjunctivae, which persisted for 7 or 48 hours, depending on concentration. The 0.1-percent formulation was tolerated without any reactions by all rabbits. Based on these results, the authors recommended that mucous membrane contact with the product be avoided (Hoechst, 1970).

In the hen’s egg chorioallantoic membrane test, isononanoic acid was evaluated as irritating (no further details; Hoechst, 1986).

7.4 Sensitisation

No information available.

7.5 Subchronic and chronic toxicity

No information available.

7.6 Genotoxicity

7.6.1 In vitro

The genotoxic potential of technical-grade isononanoic acid (3,5,5-trimethylhexanoic acid; purity not specified) was assessed in accordance

with OECD guidelines Nos. 471 and 472 as well as Directive 79/831/EEC, Annex V, 4.3.1, by means of the Salmonella/microsome assay in *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538 and tests in *Escherichia coli* WP2uvrA. Concentrations ranging from 4 to 10000 or 5000 µg/plate were tested in two independent series of tests both with and without metabolic activation (S9 mix from Aroclor 1254-induced rat liver). Bacteriotoxic effects were seen in most of the strains at concentration levels ≥ 2500 µg/plate. Isononanoic acid was mutagenic neither in the presence nor absence of metabolic activation (Hoechst, 1988).

7.6.2 In vivo

No information available.

7.7 Carcinogenicity

No information available.

7.8 Reproductive toxicity

The effect of isononanoic acid (3,5,5-trimethylhexanoic acid pure) on fertility, pregnancy and postnatal development was investigated in a one-generation study conducted in the Crl:CD®BR-VAF/Plus® rat in accordance with Directives 67/548/EEC and 88/302/EEC. Groups of 10 animals/sex and dose (males and females weighing 221 to 266 and 168 to 201 g, respectively, at treatment initiation) were fed diets containing isononanoic acid at 0 (control), 0.06, 0.12, 0.25 or 0.5% for 10 weeks before mating and during the mating period of maximum 2 weeks. After confirmation of mating (gestation day 0) the females were separated from the males and continued treatment with isononanoic acid until weaning of the offspring on postpartum day 28. Under continued administration of the chemical, pups were examined at regular intervals up to postnatal day 42 (F_1 males) or 49 (F_1 females). Relative to food consumption and body weights the parental males and females ingested isononanoic acid at 0 (control), 32, 66, 145 or 290 and 0 (control), 40, 81, 167 or 347 mg/kg body weight/day during the week before mating. There were no treatment-related deaths or clinical signs of toxicity in the parental animals. Food consumption was temporarily

reduced for males and females in the 0.5% dose group and for females in the 0.25% group, findings which the investigators considered to be due to reduced palatability of the diet. Body weights were lower than controls at gestation days 7 and 21 and postpartum days 4, 7 and 21 in females given 0.5% in their feed and at postpartum day 4 in females given 0.25%. The retardation of body weight was partially reversible at the end of the lactation period; from postpartum days 4 and 7, respectively, maternal body weights showed more marked increases than did the controls. Dietary isononanoic acid resulted in dose-related increases in relative and absolute liver weights in females fed the 0.25% diet and males and females fed the 0.5% diet. No histopathological examination was performed on the livers. Males examined after mating showed no substance-related effects on spermatid counts, progressive sperm motility or sperm morphology. Macroscopic examination and organ weight determinations for reproductive organs yielded no abnormal results for males, or for females examined on postpartum day 28 after weaning of the pups. Mating behaviour, mating efficiency, gestation and parturition also were not affected by treatment with isononanoic acid. Adverse effects in the F_1 animals were restricted to those dose groups which also exhibited parental toxicity. In the 0.5% dose group, live births were decreased by 6% and F_1 survival indices for postnatal days 1 and 4 were reduced by 14 and 21%, respectively, relative to the control. F_1 body weights were decreased in a dose-related manner compared with controls up until necropsy at postnatal days 42 or 49, with decreases being recorded at most assessment intervals for the 0.25% dose group and at all intervals for the 0.5% dose group. Also, development was slightly retarded in F_1 animals of the 0.5% dose group (delayed onset of eye opening, pinna detachment and preputial separation), a finding the investigators considered to be due to reduced body weight. Similarly to the maternal body weights, increases in F_1 body weights greater than control were noted from about the middle of the lactation period, so that body weight decreases were in fact partially reversible despite the continued administration of test substance. External examination and necropsy of F_1 animals treated with isononanoic acid revealed no treatment-related abnormalities. The *no observed adverse effect level* (NOAEL) for dietary isononanoic acid (approx. 66 and 81 mg/kg body weight/day based on parental food consumption recorded for males and females, respectively, shortly before mating) was 0.12% for both parental animals and F_1 offspring (Exxon, 1998).

In the Wistar rat, subacute oral administration of isononanoic acid at 200 mg/kg body weight/day induced no histopathological changes in the reproductive organs. Examinations were performed on the testes, epididymides, prostate gland, ovaries and uterus (see also Section 7.2; BASF, 2002).

7.9 Effects on the immune system

The immunocompetent organs and tissues (spleen, thymus, mesenteric and mandibular lymph nodes and Peyer' patches of the jejunum) of Wistar rats treated at 200 mg /kg body weight/day showed no abnormal histopathological findings in a 28-day oral study of isononanoic acid (see also Section 7.2; BASF, 2002).

7.10 Neurotoxicity

In the context of a 28-day study, Wistar rats received isononanoic acid by oral gavage at daily dose levels of 0 (control), 10, 50 or 200 mg/kg body weight and underwent comprehensive "Functional Observational Battery" (FOB) testing and motor activity assessments. There was no indication of any neurotoxic potential for isononanoic acid; the comprehensive FOB test results were unremarkable and the peripheral and central nervous system showed no abnormal histopathological findings for top-dose animals. The reduced motor activity noted in females treated at 200 mg/kg body weight was evaluated by the investigators as constituting a secondary consequence of general systemic toxicity at this level rather than a specifically neurotoxic effect (see also Section 7.2; BASF, 2002).

7.11 Other effects

No information available.

8 Experience in humans

According to the Informationsverbund Dermatologischer Kliniken (IVDK, Information Network of Departments of Dermatology for the surveillance and scientific evaluation of contact allergies in Germany), there are no data on the sensitising potential of isononanoic acid. On the basis of structure-

activity relationships and in view of the fact that isononanoic acid lacks salient structural features typical of allergens, it was deemed unlikely that the chemical has any allergenic potential (IVDK, 1997).

The occupational medicine and healthcare protection departments of two companies manufacturing and processing isononanoic acid report that after many years of experience, there is no evidence to indicate that the chemical has a sensitising potential (BASF, 1997; Hoechst, 1997).

9 Classifications and threshold limit values

The German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area ("MAK-Kommission") has listed isononanoic acid in the "Yellow Pages" ("Substances being Examined for the Establishment of MAK Values and BAT Values") of the List of MAK and BAT Values 2003 on the suggestion of BG Chemie in order that a MAK value be established for the chemical (DFG, 2003).

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