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FULLERENES (C60): SUMMARY OF THE DOSSIER

Series on the Safety of Manufactured Nanomaterials
No. 69

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OECD Environment, Health and Safety Publications

Series on the Safety of Manufactured Nanomaterials

No. 69

FULLERENES (C60): SUMMARY OF THE DOSSIER

IOMC
INTER-ORGANIZATION PROGRAMME FOR THE SOUND MANAGEMENT OF CHEMICALS
A cooperative agreement among FAO, ILO, UNDP, UNEP, UNIDO, UNITAR, WHO, World Bank and OECD

Environment Directorate
ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT
Paris, 2016

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The Environment, Health and Safety Division publishes free-of-charge documents in eleven different series: **Testing and Assessment**; **Good Laboratory Practice and Compliance Monitoring; Pesticides; Biocides; Risk Management; Harmonisation of Regulatory Oversight in Biotechnology; Safety of Novel Foods and Feeds; Chemical Accidents; Pollutant Release and Transfer Registers; Emission Scenario Documents;** and **Safety of Manufactured Nanomaterials.** More information about the Environment, Health and Safety Programme and EHS publications is available on the OECD's World Wide Web site (www.oecd.org/chemicalsafety/).

This publication was developed in the IOMC context. The contents do not necessarily reflect the views or stated policies of individual IOMC Participating Organizations.

The Inter-Organisation Programme for the Sound Management of Chemicals (IOMC) was established in 1995 following recommendations made by the 1992 UN Conference on Environment and Development to strengthen co-operation and increase international co-ordination in the field of chemical safety. The Participating Organisations are FAO, ILO, UNDP, UNEP, UNIDO, UNITAR, WHO, World Bank and OECD. The purpose of the IOMC is to promote co-ordination of the policies and activities pursued by the Participating Organisations, jointly or separately, to achieve the sound management of chemicals in relation to human health and the environment.

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1. IDENTITY

1.1. Identification of the Substance

CAS Number:	9968-96-8
IUPAC Name:	
Molecular Formula:	C_{60}
Structural Formula:	
Molecular Weight:	
Tested Materials:	Nanom purple (Frontier Carbon Co.) Fullerenes (Mer Corp.)

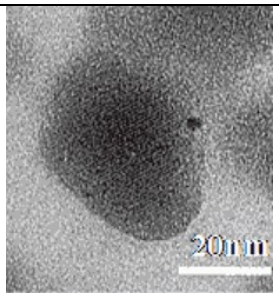
1.2. Purity/Impurities/Additives

Materials	Purity
Nanom Purple (Frontier Carbon Co.)	>99.8 % (w/w)
C60 (MER Corp.)	>99% (w/w)

1.3. Physical-Chemical Properties

Table 1. Summary of Physical-Chemical Properties *(Data of* ***Nanom Purple*** *except for some read across data)*

Endpoint	Results	method
Agglomeration/aggregation[1]	Primary particles of around 30 nm dia. were aggregated or agglomerated in a size of about 500 nm or larger	Observed by TEM
Water solubility[2]	$1.3x10^{-11}$ mg/l (extrapolation of the solubility study results in several alcohols)	Read across (C_{60} from Bucky USA) Heymann D. et al.
Crystalline phase[3]	fcc (face-centered cubic) above 260K and sc (simple cubic) beneath 260K.	Read across Fischer. et al
Dustiness[4]	0.65 mg/m^3 (GSD=1.6) (respirable particle)	Vortex Shaker Method (Maynard 2004)
Crystallite size[1]	The medium mass diameter ranged from 20 to 30 nm	Observed by TEM
Representative TEM picture[1]		

		
Particle size distribution[5]	The measured medium mass diameter ranged from 20 to 30 nm.	DLS
Specific surface area	0.87 m^2/g.	ISO 9277 (BET method)
Zeta potential	-20mV Sample used for mammalian toxic. test	electrophoretic mobility method
Surface chemistry	Not relevant	
Photocatalytic activity[6]	No photocatalytic activity	ISO 22197-1 / JIS R 1701
Pour density[6]	0.838 g/cm^3	ASTM D 1513-05
Porosity[6]	Pore volume = 0.52 ml/g Davg = 88 nm	ISO 15901-1
Octanol-water partition coefficient[7]	Kow = 6.67	Read across (C_{60} from MER Corp.) Jafvert C.T. et al.
Redox potential		
Radical formation potential including surface properties	---	The method is not specified.

2. GENERAL INFORMATION ON EXPOSURE

2.1 Environmental Exposure and Fate

2.1.1. Photodegradation

No information is available.

2.1.2. Stability in Water

No information is available

2.1.3. Transport between Environmental Compartments

No information is available.

2.1.4. Biodegradation

No information is available.

2.1.5. Abiotic Degradability and Fate

Three phototransformation studies using Fullerenes supplied by **MER Corporation** were conducted.

Isaacson et al.[8] studied the formation of aqu/C_{60} (fullerenes fully suspended in water) in various media. 100 mg of C_{60} powder (Supplied by **MER Corp.**) was added to 400 ml of deionized (DI) water, SRHA (Swannee River Humic Acid) solutions (0.1, 1.0 and10 mg/L) and Calls Creek water from Athens, Ga. Formation of aqu C_{60} were examined by comparing in the dark and in the natural sun exposure (450 W/m2). As a result, all dark treatments except the Calls Creek treatment had increased concentrations of aqu/C_{60} over the course of a 200 hours suspension time period. All aqu/C_{60} suspensions formed under sunlight exhibited a trend where concentrations increased for the first 100 hours of suspension time and then maintained a constant concentration over the next 100 hours. The results demonstrate that sunlight and humic acid have significant effects on the concentration, size and zeta potential of the aqu/C_{60}. Sunlight is clearly an important determinant of the mass of aqu/C_{60} available in the water column since sunlight increased the suspended mass of aqu/C_{60} in water from a surface stream by over an order of magnitude.

Kong et al.[9] studied the effect of the sun light using the solar simulator according to OECD TG 316. To prepare aqueous C_{60} clusters (nC_{60}), Aqueous suspensions of C_{60} were prepared by mixing ground C_{60} (supplied by **MER corp.**) powder (400 mg) with Nanopure water (1 L) on a magnetic stirrer for at least four weeks. The incident irradiance at the tube surface, summed from 290 to 700 nm, was 0.0065 W cm^{-2}. Monochromatic irradiation experiments were conducted in 8 mL Pyrex glass tubes that each contained 5.00 mL of solution. After the radiation, the production of singlet oxygen by nC_{60} is accelerated by increasing oxygen concentration and in part is sensitized by fluorescent photoproducts that accumulate during irradiation. These results show that environmental conditions, including light exposure and oxygen concentration, have the potential to impact the generation of toxic ROS by fullerenes.

Hou et al.[10] also studied the phototransformation of C_{60} (**MER Corp.**) under natural sunlight according to OECD TG 316. To prepare aqueous C_{60} clusters (nC_{60}), 80 mg C_{60} dry powder was magnetically stirred in pure water (1 L) for about 2 weeks prior to passing it though 0.7 and 0.45 um membrane filters. All solar irradiations occurred either on the roof of the Civil Engineering Building at Purdue University or on a nearby farm field. In experiments that examined wavelength dependent effects, 280 or 400 nm cutoff filters were used. The experiments showed that long-wavelength light ($\lambda \approx 400$ nm) isolated from sunlight, was shown to be important in both the phototransformation of nC_{60} and in the production of singlet oxygen. Photoproducts have olefinic carbon atoms as well as a variety of oxygen-containing functional groups, including vinyl ether and carbonyl or carboxyl groups, whose presence destroys the native pi-electron system of C_{60}. Findings from this work suggest that C_{60} is unlikely to persist in sunlit aquatic environment (half life =~ 1 day in noon sunlight). Sunlight exposure leads to hydroxylated and carboxylated C_{60} photoproducts with structure similar to fullerol (i.e., polyhydroxylated C_{60}).

2.1.6. Bioaccumulation

Patras et al. [11] studied on the bioaccumulation of fullerenes from **MER Corp.** The crustacean *Thamnocephalus platyurus* was exposed to aqueous suspensions of fullerenes C_{60}. Aqueous fullerene suspensions were formed by stirring C_{60} in deionized water (termed aqu/C_{60}) for 100 days. The Z-average (mean hydrodynamic) diameters of aqu/C_{60} aggregates as measured by DLS was 517 ± 21 nm. Exposure of fullerene suspensions to T. platyurus resulted in the formation of dark masses in the digestive track visible under a stereo microscope (×40 magnification). Fullerene ingestion over 1 h of exposure was quantitatively determined after extraction and analysis by high-performance liquid chromatography-mass spectrometry (HPLC-MS). The uptake of aqu/C_{60} between 0 and 10 minutes was rapid for both concentrations of 3 mg/L and 6 mg/L. However, after the initial uptake period, the C_{60} concentration per unit mass of organism did not change significantly over the remaining 1 hour time period. One-hour exposures (at 3 mg/L and 6 mg/L) resulted in aqu/C_{60} burdens of 2.7 ± 0.4 μg/mg and 6.8 ± 1.5 μg/mg wet weight, respectively. Thin-section TEM images of aqu/C_{60}-exposed T. platyurus showed the formation in the gut of fullerene agglomerates (5–10 μm) that were an order of magnitude larger than the suspended fullerene agglomerates. Upon excretion, the observed fullerene agglomerates were in the 10 – 70 μm size

range and settled to the bottom of the incubation wells. In contrast to the control polystyrene microspheres, which dispersed after depuration, the aqu/C_{60} agglomerates (greater than two orders of magnitude larger than the suspended fullerenes) remained agglomerated for up to six months. When exposed to fullerenes, T. platyurus shows the potential to influence agglomerate size and may facilitate movement of these nanoparticles from the water column into sediment.

2.1.7. Adsorption to soil and sediment

Two studies are available about adsorption to soil and sediment.

The first study was conducted by Isaacson et al.[12] in 2011. 100 mg of C_{60} powder supplied by **Mer Corp.** was added to 400 mL of deionized (DI) water and then stirred on magnetic stir plates under ambient laboratory lighting. Subsamples of aqu/C_{60} (fullerenes in a completely aqueous system) suspensions were collected from three different suspensions that were stirred for approximately 99, 309 and 1075 days (i.e. short, intermediate and long intervals). As suspension time increased, aqu/C_{60} particle size shifted to a large size range as determined by AF4. Long term aqu/C_{60} ranged in size from 240-410 nm in hydrodynamic diameter while short term aqu/C_{60} ranged from 220-360 nm in hydrodynamic diameter. Quart crystal microbalance (QCM) deposition measurements indicate that the short term aqu/C_{60} was readily deposited on the SiO_2 surface while intermediate term aqu/C_{60} was less readily deposited and no deposition was observed for long term stirred aqu/C_{60}. All three aqu/C_{60} suspensions were poorly retained in the saturated sand columns as the average mass recovered in the effluent ranged from 78 to 94%, indicating the aqu/C_{60} has the potential to travel significant distances upon entering into groundwater aquifers or river sediments.

The second study was conducted by Zhang et al.[13] in 2012. Bulk aqu/C_{60} (**Mer Corp.**) suspensions were generated by stirring into DI water under ambient laboratory lighting for over 3 years, resulting in less hydrophobic aqu/C_{60} nanoparticles. Bulk aqu/C_{60}-PTA (pyrrolidine tris-acid) suspensions were generated by stirring in DI water for only two months. Two model porous media, high purity Iota quartz (99.9% silica) and Ottawa sand (99.9% silica) and freshwater sediment collected from Calls creek near Athens, GA. Aqu/C_{60} suspensions consisted of aggregates with a mean size of 172-195 nm. The AF4 analyses indicate that C_{60}-PTA in DI water were much smaller than aqu/C_{60}. The aqu/C_{60} and C_{60}-PTA had the least retention in Iota quartz, and the greatest retention in the sediment at near neutral pH and 10 mM ion strength in varying media type, correlating with the degree of grain surface chemical heterogeneity. In general, aqu/C_{60} was more retained than C_{60}-PTA at near neutral pH, due to the difference in interaction energies. Aqu/C_{60} and C_{60}-PTA both exhibited pH-dependent transport behaviors (a slight decrease in retention when solution pH was increased to 10 and a significant increase in retention when solution pH was lowered to 4 even at lower IS of 1.5 mM). Because fullerene retention was greater in the tested river sediment than in model porous media, fullerene nanomaterials may be more attenuated in some types of soils, river sediments, or groundwater aquifer than in model porous media (e.g. clean glass beads or quartz sand).

2.2. Summary

Phototransformations of **MER C_{60}** were tested by irradiation of natural and/or artificial sunlight. The aqu/C_{60} (fullerenes fully suspended in water) was exposed to natural sunlight in various media and the sun light increased the suspended mass of aqu/C_{60} in water. The tests according OECD TG 316 under artificial and natural sunlight show that C_{60} is unlikely to persist in sunlit aquatic environment (half life =~ 1 day in noon sunlight) and produces ROS.

For studying the bioaccumulation, *T. platyurus were exposed to* **MER C_{60}** After One-hour exposures (at 3 mg/L and 6 mg/L), the burden of C_{60} was 2.7 ± 0.4 µg/mg and 6.8 ± 1.5 µg/mg wet weight, respectively. C_{60} uptake and subsequent excretion was found to form stable and greater agglomerations which may facilitate movement of these nanoparticles from the water column into sediment.

The adsorptions of **MER C_{60}** to soil and sediment were examined in two studies. Both studies showed the adsorption to soils and indicated the potential to travel significant distances upon entering into groundwater aquifers or river sediments.

3. HAZARDS TO THE ENVIRONMENT

3.1. Aquatic Effects

Acute Toxicity Test Results

Fish

One study is available on the acute toxicity to fish.

Shinohara et al. (2011)[14] studied the effects of fullerenes (**Nanom Purple**, Frontier Carbon Corp.) on acute toxicity to the fish Cyprinus carpio under GLP, following OECD Test Guideline 203. Seven individuals in each group were exposed in static system at nominal concentrations of 4.5 mg/L of fullerene suspension with a blank water (control) for 48 hours. Tween 80 of 0.1% was used as a vehicle for the preparation of the test solution. Neither mortality nor toxicological symptoms were observed at the concentration of 4.5 mg/L after 48 hours exposure. Further the LPO (Lipid Peroxididation) concentrations of the extracted brain samples were measured under irradiation to investigate the translocation of C60 to the brain. No LPO concentration increase was observed and this indicated very few or no C60 nanoparticles reached the brain of C. carpio.

Aquatic Invertebrates

No information is available.

Aquatic plants

No information is available.

Chronic Toxicity Test Results

Fish

One reference study is available on the dietary toxicity to aquatic vertebrates.

Fraser, et al. (2011)[15] exposed juvenile female trout, *Oncorhynchus mykiss* (45 fish/tank in triplicate design) to fullerenes in feed at a concentration of 500 mg/kg for six weeks and monitored for mortality, growth, blood parameters, tissue alterations, and changes in biochemical parameters. The fullerenes (purity: 99.9%) were obtained from **SES Research**. Dietary exposure to fullerenes had no significant effect on growth, hematology, or other measured endpoints in trout. Lipid peroxidation, as indicated by changes in thiobarbituric acid reactive substance (TBARS) measurements, was not also significantly different from that of the fish control. It was noted that longer duration studies, other fish species, and additional endpoints (such as bioenergetics and swimming speed) have not been examined.

Aquatic Invertebrates

No information is available.

Toxicity to Microorganisms

No information is available.

3.2. Terrestrial Effects

No information is available.

3.3. Summary

The acute toxicity of fullerenes (**Nanom Purple**) to the fish Cyprinus carpio was tested following OECD Test Guideline 203. Neither mortality nor toxicological symptoms were observed at the concentration of 4.5 mg/L after 48 hours exposure. Further the LPO concentrations of the extracted brain samples were measured under irradiation and no LPO concentration increase was observed. This indicated that very few or no C60 nanoparticles reached the brain.

The dietary exposure of fullerenes (**SES Research**) to juvenile female trout (*Oncorhynchus mykiss*) was studied by feeding 500 mg/kg of fullerenes for six weeks. No significant effect on growth, hemotology, or other measured endpoints in trout was observed.

4. HUMAN HEALTH HAZARDS

4.1. Effects on Human Health

4.1.1. Toxicokinetics

Studies in Animals

In vivo Studies

To estimate the clearance rate and deposition fraction of C_{60} from inhalation exposure, the C_{60} burden in the lungs, liver and brain of rats was determined after intratracheal instillation and inhalation (Shinohara *et al.* 2010)[16]. Male Wistar rats (6 rats/dose/observation period) were intratracheally instilled of a C_{60} (**Nanom Purple)** suspension prepared with Tween 80 at the dose of 0.1, 0.2 and 1 mg/rat or exposed to a C_{60} aerosol prepared with nebulizer at a concentration of 0.12 mg/m^3. C_{60} burdens in the lungs, liver and brain was determined at various points (1h to 6 months) by sensitive high-performance liquid chromatography-ultraviolet absorptiometry. Inhaled C_{60} clearance from the lung was evaluated using a 2-compartment model; fast clearance after deposition on lung surface and slow clearance after retention in the epithelium. Pulmonary C_{60} burden decreased with time and depend on the C_{60} concentration administered. The concentration of C_{60} in the liver and brain was below the detection limit: 8.9 ng/g tissue after intratracheal instillation and inhalation. The half-life in the lung of intratracheally instilled C_{60} was 15-28 days. Mode evaluation revealed that most instilled particles could be eliminated by the fast clearance pathway. This finding was consistent with the transmission electron microscopy finding that many particles were present in alveolar macrophages.

Biodistribution of C_{60} (**Nanom Purple)** in male wister rats (5 rats/time point) after tail vein administration (5 mg/kg bw/injection x 4 times) was examined using LC-MS/MS (Kubota *et al.* 2011)[17]. C_{60} was detected in various tissues, such as brain, kidneys, liver, lungs, and spleen of male Wistar rats. On the other hand, no C_{60} was found in blood. The highest C_{60} concentration was observed in the lungs, followed by spleen, liver, kidneys and brain. These results suggested that C_{60} injected in the tail vein could be filtered by lung capillary vessels and accumulate in the lungs prior to being distributed to other tissues. Furthermore, C_{60} not being detected in the blood indicated that clearance of C_{60} from the blood by filtration might effectively occur in the lungs. The time-dependent variation in the biodistribution of C_{60} was evaluated. A time-dependent decrease in C_{60} concentrations was observed in all tissues, except spleen. Moreover, a decreasing trend of C_{60} levels differed among tissues, which could be due to differences in accumulation.

Studies in Humans

No information is available.

4.1.2. Acute Toxicity

Studies in Animals

Inhalation

An acute inhalation toxicity study is not available; however, in a repeated inhalation test of fullerene C_{60}nanoparticles (**Nanom Purple)** aerosol, no deaths were observed at 0.12 mg/m^3 (see 4.1.5 repeated dose toxicity for further detail). Based on this finding, the LC_{50} for inhalation toxicity was considered to

be greater than 0.12 mg/m^3 in rats. Moreover, in a single intratracheal instillation test using rats dosed up to 3.3 mg/kg bw, no deaths were observed (see 4.1.9 additional information for further detail).

Dermal

An acute dermal toxicity study is not available; however, in two skin irritation studies, no deaths or abnormal findings were observed in male rabbits applied 0.5 g of fullerene C_{60} (**Nanom Purple)** with 0.5 g of olive oil, 0.02 g fullerene wetted with 0.2 ml of vehicle (direct application) or 0.5 ml of 20% fullerene C_{60} suspension (see 4.1.3 skin irritation for further detail). Acute dermal toxicity was considered to be low.

Oral

An acute oral toxicity study is not available; however, in a repeated oral test of C_{60} (**Nanom Purple)** nanoparticles, no deaths were observed up to 1000 mg/kg bw/day (the highest dose) (see 4.1.5 repeated dose toxicity for further detail). Based on this finding, the LD_{50} for oral toxicity was considered to be greater than 1000 mg/kg-bw in rats. Moreover, in an *in vivo* micronucleus study of C_{60} nanoparticles using mice, no death or indicative of abnormality was observed up to 88 mg/kg bw/day (the highest dose) (see 4.1.6 mutagenicity for further detail).

Studies in Humans

No information is available.

4.1.3. Irritation

Skin Irritation

Studies in Animals

Two skin irritation studies were conducted according to OECD TG 404.

In one study, 0.5 g of fullerene C_{60} (**Nanom Purple)** with 0.5 g of olive oil (vehicle) was applied to three male New Zealand White rabbits of 17 weeks of age (Matsuda *et al.* 2010a)[18]. The test substance was occlusively applied for 4 hours at the skin of the back with patch. At 1, 24, 48 and 72 hrs after removal of the patch, application sites were observed. No indication of skin irritancy was observed for all test sites in all rabbits through the observation period.

In the other study, 0.02 g fullerene (**Nanom Purple)** wetted with 0.2 ml of vehicle (5% gum arabic solution) (direct application) or 0.5 ml of 20% fullerene C60 suspension was applied to each three male JW rabbits of 10 weeks of age (Kawabe et al. 2011a)[19]. The test substance was occlusively applied for 4 hours at the back skin of the animals. At 1, 24, 48 and 72 hrs after removal of the patch, application sites were observed. In either exposure method of direct application or suspension, fullerene did not cause both erythema and edema in rabbits during 72 hours after exposure. Primary irritation index (PII) was, thus, calculates as 0.0.

Studies in Humans

No information is available.

Eye Irritation

Studies in Animals

An eye irritation test was done using three male New Zealand White rabbits for fullerene C_{60} (**Nanom Purple)** following OECD TG 405 (Matsuda *et al.* 2010b)[20]. 0.1 g of the test material was applied in conjunctiva sac of the left eye and the eyelid was gently closed for one second. The right eye was untreated and served as a control. At 1, 24, 48 and 72 hours after treatment, the irritating reactions of the eyes were observed. At 1 hr after exposure, redness of conjunctivae (score = 1) was observed in the treated eyes of all three rabbits, but recovered within 24 hrs. No other changes were observed in any animals during the observation period.

Studies in Humans

No information is available.

Respiratory Tract Irritation

Studies in Animals

Studies in Humans

No information is available.

4.1.4. Sensitization

Studies in Animals

Skin

Two sensitization studies were conducted according to OECD TG 406.

One sensitization test was executed using 40 male Hartley guinea pigs (10 for the negative control, 10 for the positive control and 20 for material treated groups) for fullerene C_{60} (**Nanom Purple)** at doses of 0.4 ml olive oil containing 10% C_{60} for induction and 0.2g of vaseline containing 10% C_{60} for challenging.(Matsuda *et al.* 2010c)[21]. No clinical signs were observed in any group. No erythema or edema was observed after the challenge. The skin sensitization experiments were properly performed, because positive dermal responses were observed in the area challenged with DNCB (dinitrochlorobenzene, positive control), but not in those areas challenged with acetone (negative control).

The other sensitization test (Kawabe *et al.* 2011b)[22] was conducted using male Hartley guinea pigs (10 for the negative control and 20 for material treated groups) for fullerene C_{60} (**Nanom Purple)**. No clinical signs were observed in the negative control and material treated groups. No erythema or edema was observed after the challenge with 20% fullerene C_{60} in 5% gum arabic. Positive dermal responses were observed in the area challenged with DNCB, although the positive control test was conducted separately from the main study.

Respiratory Tract

No information is available.

4.1.5. Repeated Dose Toxicity

Studies in Animals

Inhalation

An inhalation test of fullerene C_{60} nanoparticles (**Nanom Purple)** aerosol was performed in male Wistar rats for 4 weeks (6 hours/day, 5 days/week, 10 rats/observation period) at a level of 0.12 +/- 0.03 mg/m^3 of the particle weight concentration in the exposure chamber (Morimoto *et al.* 2010[23], Ogami *et al.* 2011[24], Fujita *et al.* 2009[25]). Animals were sacrificed at 3 days, 1 month and 3 months after the end of exposure. Based on data for histopathological examination, BALF (bronco-alveolar lavage fluid) examination, chemokine analysis in lung tissue and DNA microarray analysis, it was suggested that C_{60} fullerene might not have a severe pulmonary toxicity after 4 weeks inhalation exposure in rats. Although slight inflammatory response was observed in the lungs, no histopathological abnormalities were observed in the liver, kidney, spleen, cerebrum, cerebellum, testis, or nasal cavity tissues in C_{60} inhalation group.

Intratracheal

No information is available.

Pharyngeal aspiration

No information is available.

Dermal

No information is available.

Oral

A repeated oral dose toxicity study was conducted based on OECD TG407 (Doi *et al.* 2011[26], Takahashi *et al.* 2012[27]). Male and Female Crl:CD(SD) rats (5 or 10 animals/sex/dose) were administered fullerene C_{60} nanoparticles (**Nanom Purple,** suspended in corn oil) by gavage at a dose of 0 (control), 1, 10, 100, 1000 mg/kg bw/day for 29 days with a 14-day recovery period (0 and 1000 mg/kg bw/day groups). After start of treatment, blackish feces were observed in male and female 1000 mg/kg bw/day group and at the necropsy, black content of stomach and large intestine were observed in the same groups. It was considered that a large amount of fullerene was contained in contents of intestine and feces. Treatment-related possible effects were observed at 1000 mg/kg bw/day in clinical chemistry (a decrease in albumin in males only at the end of administration period and an increase in total protein in females only at the end of recovery period) and some organ weights (a decrease in kidney weight in males only at the end of administration period, and increases in liver and spleen weights in males only at the end of recovery period), but these were slight and not accompanied with histopathological changes, and then NOAEL was determined to be 1000 mg/kg bw/day (the highest dose tested).

Studies in Humans

No information is available.

4.1.6. Mutagenicity

Studies in Animals

In vitro Studies

Bacterial mutation

A reverse gene mutation assay for fullerene C_{60} nanoparticles (**Nanom Purple,** suspended in CMC-Na, carboxymethyl cellulose-Na, solution) was performed using *S. typhimurium* TA98, TA100, TA1535 and TA1537 and *E. coli* WP2*uvrA*/pKM101 according to OECD TG471 and the Japanese Guideline (Chemical Substances Control Law of Japan) at the concentration of 50, 100, 200, 400 and 1000 μg/plate under dark or irradiation with visible light condition (Matsumoto *et al.* 2008b[28], 2008c[29], Shinohara *et al.* 2009[30]). Regardless of metabolic activation and irradiation, the growth inhibition was not found in all strains and dose amounts. Although the precipitation of the test material was not seen with 50, 100, 200 μg/plate, that with 400, 1000 μg/plate was difficult to judge because the sample solution was black. However, clear precipitation was seen at the 1000 μg/plate dose in the test with irradiation.

More than double of the reverse mutation colonies was not found comparing with the negative control group in any strains and dose levels tested, regardless of metabolic activation and irradiation. It was concluded that in vitro bacterial mutagenicity of fullerene C_{60} nanoparticles is negative regardless of metabolic activation and irradiation. Positive control showed expected levels of mutagenicity.

Chromosomal aberration

A chromosomal aberration test for fullerene C_{60} nanoparticles (**Nanom Purple,** suspended in CMC-Na solution) was conducted using cultured Chinese hamster lung (CHL/IU) cells according to the OECD TG 473 and Japanese Guideline (Chemical Substances Control Law of Japan) (Matsumoto *et al.* 2008d[31], 2008e[32], Shinohara *et al.* 2009[30]). In a preliminary test, precipitation of C_{60} occurred at a concentration of 85 μg/mL without irradiation or 200 μg/mL with irradiation after treatment. Test concentration used in the chromosomal aberration test (i): Short-term treatment assay; under dark conditions, the concentrations used were 12.5, 25, 50 and 100 μg/mL without S9 mix, and 25, 50 and 100 μg/mL with S9 mix, respectively, and under irradiation, the concentration used were 50, 100 and 200 μg/mL without S9 mix, and 25, 50, 100 and 200 μg/mL with S9 mix, respectively. 0.1% CMC-Na was used as a negative control, and mitomycin C (MMC), benzo[a]pyrene (B[a]P) or acridine orange (AO) were used as positive controls. Test concentration used in the chromosomal aberration test (ii): Continuous treatment assay; under dark conditions, the test concentrations used were 12.5, 25, 50 and 100 μg/mL without S9 mix, and under irradiation, 25, 50, 100 and 200 μg/mL without S9 mix, respectively. 0.1% CMC-Na was used as a negative control, and MMC, B[a]P or AO were used as positive controls.

In either test condition, the expressions of structural chromosomal aberration and polyploidy were below 5%. Cytotoxicity was not observed up to the highest concentration. The positive controls were effective for induction of chromosome aberrations. It was concluded that *in vitro* chromosomal aberration test of fullerene C_{60} nanoparticles is negative regardless of metabolic activation and irradiation.

In vivo Studies

Chromosomal aberration

Fullerene C_{60} nanoparticles (**Nanom Purple,** suspended in Tween 80) was administrated twice by gavage in the interval of 24 hours to male ICR mice of the age of 8 weeks (5 mice a group) at 0, 22, 45 and 88 mg/kg/day (Matsumoto *et al.* 2008a[33], Shinohara *et al.* 2009[30]). The available highest concentration of C_{60} in 0.1% Tween80 solution was approximately 4.4 mg; therefore, 88 mg/kg was set as the highest dose in this test. In 24 hours, the bone-marrow cell was harvested from the femur and

micronucleus assay was performed following OECD TG 474. No abnormality was seen in all the group of mice and no micronucleus formation was observed in each dose group.

DNA damage and/or repair

Fullerene C_{60} nanoparticles (**Nanom Purple)** suspension (in Tween 80) was dosed at 0, 0.5 or 2.5 mg/kg once or 0, 0.1 or 0.5 mg/kg for 5 times (once/week) by intratracheal administration in male Crl:CD (SD) rats (Ema *et al.* 2012)[34]. A comet assay was conducted in lung tissue taken from 3 h (single dose and repeated dose) or 24 h (single dose) after the (last) administration. There were no effects on percentile DNA. It was concluded that fullerene C_{60} nanoparticles had no potential for DNA damage in comet assays using the lung cells of rats given fullerene C_{60} nanoparticles even at doses causing inflammation.

Studies in Humans

No information is available.

4.1.7. Carcinogenicity

No tumors or granulomas were observed in the repeated inhalation study and the intratracheal instillation study (Ogami *et al.* 2011)[16]. See 4.1.2 and 4.1.9 for further detail.

4.1.8. Toxicity for Reproduction

No information is available.

4.1.9. Additional Information

Fullerene C_{60} nanoparticles (**Nanom Purple**, suspended in distilled water with 1% Tween 80) were administrated to male Wistar rats by single intratracheal instillation at 0, 0.1, 0.2, and 1 mg/rat (equivalent to 0 , 0.33, 0.66, and 3.3 mg/kg bw) (Morimoto *et al.* 2010[23], Ogami *et al.* 2011[24], Fujita *et al.* 2010[35]). After a single intratracheal instillation, the rats of control and treatment groups were sacrificed at 3 days, 1 week, 1 month, 3 months, 6 months and 12 months after the last exposure. Based on data for histopathological examination, BALF examination, chemokine analysis in lung tissue and DNA microarray analysis, it was concluded that fullerene C_{60} intratracheally instilled did not show an obvious pulmonary inflammation up to the dose of 0.2 mg in rats (0.66 mg/kg bw). Fullerene C_{60} at 1 mg (3.3 mg/kg bw) revealed an obvious inflammatory response in the lungs; accumulations of macrophages and inflammatory cells of the lungs were observed only at 1 mg at 12 months after instillation.

In vitro tests for fullerene C_{60} nanoparticles (**Nanom Purple)** using three kinds of cell lines; human lung adenocarcinoma cell line (A549); human keratinocyte cell line (HaCaT); and human acute monocytic leukemia cell line (THP-1) were executed to examine cell viability, oxidative stress and apoptosis as endpoints (Horie *et al.* 2010)[36]. In conclusion, fullerene C_{60} nanoparticles did not influence viabilities and LDH activities of HaCaT and A549 cells. Apoptosis was not increased but cell proliferation rate slightly decreased. The oxidative stress marker and DNA damage were observed. There was no evidence that fullerene C_{60} nanoparticles will cause adverse effect, but possibility of the long-term effect due to the DNA damage cannot be denied.

4.2 Summary

The test results of Human Health Hazards of **Nanom Purple C60** are summarized below.

Toxicokinetics

To estimate the clearance rate and deposition fraction of C_{60}, inhalation exposure and intratracheal instillation tests to male Wistar rats were conducted. Pulmonary C_{60} burden decreased with time and depend on the C_{60} concentration administered. The concentration of C_{60} in the liver and brain was below the detection limit after intratracheal instillation and inhalation. The half-life in the lung of intratracheally instilled C_{60} was 15-28 days.

Biodistribution of C_{60} in rats after tail vein administration was examined. C_{60} was detected in various tissues with the highest concentration in lungs, but not in blood. C_{60} injected in the tail vein could be filtered by lung capillary vessels and accumulated in the lungs prior to being distributed to other tissues. The clearance of C_{60} from the blood by filtration was fast. A time-dependent decrease in C_{60} concentrations was observed in all tissues, except spleen.

Acute toxicity

Though there is no specific information on acute toxicity, some of toxicity test results are available as supporting information.

In a repeated inhalation test of fullerene C60 nanoparticles aerosol, no deaths were observed at 0.12 mg/m^3.

In two skin irritation studies, no deaths or abnormal findings were observed in male rabbits

In a repeated oral test of C60 nanoparticles, no deaths were observed up to 1000 mg/kg bw/day.

Irritation

Two skin irritation studies were conducted in New Zealand White rabbits or JW rabbits, and fullerene did not cause erythema nor edema during 72 hours after exposure in the both studies.

An eye irritation test were done using three male New Zealand White rabbits for fullerene C60 following OECD TG 405. At 1 hr after exposure, redness of conjunctivae was observed in the treated eyes of all three rabbits, but recovered within 24 hrs.

Sensitization

Two skin sensitization studies were conducted according to OECD TG 406 using male Hartley guinea pigs. No erythema or edema was observed after the challenge in both studies.

Repeated dose toxicity

A repeated dose inhalation test of fullerene C60 nanoparticles aerosol was performed in male Wistar rats for 4 weeks (6 hours/day, 5 days/week) at a concentration of 0.12 mg/m^3. Based on data for histopathological examination, BALF (bronco-alveolar lavage fluid) examination, chemokine analysis in lung tissue and DNA microarray analysis, it was suggested that C60 fullerene might not have a severe pulmonary toxicity after 4 weeks inhalation exposure in rats. Although slight inflammatory response was observed in the lungs, no histopathological abnormalities were observed in other organs.

A repeated oral dose toxicity study was conducted based on OECD TG407 using male and female Crl:CD(SD) rats at a dose up to 1000 mg/kg bw/day for 29 days. Treatment-related effects were observed at 1000 mg/kg bw/day in clinical chemistry and some organ weights, but these were slight and not accompanied with histopathological changes.

Mutagenicity

An in vitro reverse gene mutation assay (OECD TG471) and an in vitro chromosomal aberration test (OECD TG 473) for fullerene C60 nanoparticles were negative regardless of metabolic activation and irradiation.

An in vivo chromosomal aberration test for Fullerene C60 nanoparticles according to OECD TG 474 was conducted. No abnormality was seen in all the group of mice and no micronucleus formation was observed.

A comet assay was conducted in lung tissue taken from rats which was intratracheal administrated fullerene C60 nanoparticles suspension. Fullerene C60 nanoparticles had no potential for DNA damage in comet assays

Carcinogenicity

No tumors or granulomas were observed in the repeated inhalation study and the intratracheal instillation study.

Additional Information

Fullerene C60 nanoparticles were exposed to male Wistar rats by single intratracheal instillation. Fullerene C60 at 1 mg (3.3 mg/kg bw) revealed an obvious inflammatory response in the lungs; accumulations of macrophages and inflammatory cells of the lungs were observed only at 1 mg at 12 months after instillation.

In vitro tests for fullerene C60 nanoparticles using three kinds of human cell lines were executed to examine cell viability, oxidative stress and apoptosis. Apoptosis was not increased but cell proliferation rate slightly decreased. The oxidative stress marker and DNA damage were observed.

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