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TESTING THE EFFECT OF FULLERENE AND ITS DERIVATIVES ON THE ENVIRONMENT

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Research article

Abstract: The paper points to the increasing use of products containing nanomaterials and warns that the public which uses such products often fails to be adequately informed about their potential hazards. The specific subject of the paper is fullerene and its bromo derivative. The contradictory opinions regarding the hazards associated with this material are discussed, and its potential environmental impacts are investigated experimentally.

Keywords: Nanomaterials, Fullerene, Fullerene bromo derivative, Hazards, Risk.

Introduction

Nanotechnology is a field of science and technology which uses the findings and methods of many classical fields such as electronics, physics, quantum mechanics, chemistry and biochemistry. Nanomaterials are diverse, as are the areas of their application, and nowadays nanomaterials are used rather widely. Research in this area focuses on the structure of nanomaterials and their behaviour on the atomic scale. Interactions between microparticles and quantum phenomena are starting to play an important role in this.

Increased attention should be paid to the safety of the manufacture and use of nanomaterials. This field has been advancing at such a fast pace that the hazardous properties of the materials so far could not be evaluated or adequate provisions adopted in the Czech Republic or in the European Union. Existing legislation applicable to nanoparticles is based on EU regulations and on laws covering chemical substances.

According to information presented in the communication "Regulatory Aspects of Nanomaterials" (Communication, 2008), any nanomaterial must meet the requirements of the REACH Regulation. Although the Regulation contains no paragraph devoted specifically to nanomaterials, they are included in the definition of a "substance". The main aim of REACH is to ensure a high level of protection of human health and the environment from the risks that can be posed by chemicals (REACH Regulation, 2006). From the above it is clear that considerable attention should be paid to the potential hazards and risks to humans and the environment arising from nanomaterials. The European Community Strategy for Health and Safety at Work has assigned several tasks, one of them being to identify health hazards associated with the use of new materials and processes (Skřehot and Rupová, 2009). Among new materials which are used in industrial processes are **fullerene and its bromo derivative**.

Fullerenes are giant molecules containing 20 or more carbon atoms in the apexes of polyhedra of spherical shape. A molecule of the most stable existing fullerene (Fig. 1) contains 60 carbon atoms and its diameter is about 1 nm. This is the most spherical and most symmetrical fullerene. All carbon atoms in the molecule are equivalent, which implies that strains are uniformly distributed throughout the structure. Its geometry is a truncated icosahedron, where 12 pentagons and 20 hexagons are so arranged that no two pentagons are neighbours (Barabaszová, 2006). This chemically best known fullerene is symbolized as C_{60}.

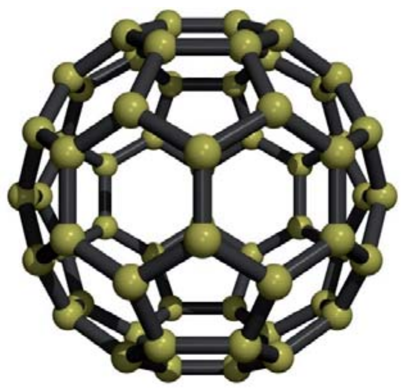

Fig. 1 Fullerene with 60 carbon atoms (C_{60}, 2004)

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Identified fullerenes whose weight is greater than C_{60} include fullerenes C_{70}, C_{76}, C_{C78}, C_{80}, C_{82}, and C_{84}. The frequency of occurrence of fullerenes with more carbon atoms than 70 decreases. The C_{60} molecule exists in a single structure whereas higher fullerenes possess different structures in their molecules (Barabaszová, 2006).

Materials and methods

Natural occurrence and manufacture of fullerene

Fullerene has been identified in space and in the cold atmospheres of meteorites. Fullerenes share 0.3 % to 0.9 % of interstellar carbon. Fullerenes exist on Earth as well. They are found in fulgurites (they emerge as a result of lightning striking soil, sand or solid rock, inducing remelting and vitrification), in volcanic craters, in boundary sediments, in solid bitumens in the graphite deposit at Mílové (Blovice area), in bituminous coal seams in China, and the like. Fullerene deposits were identified in the mineral called shungite, which is found in north-western Russia near the Finnish border (Karelia region) (Klouda and Kubátová, 2010). It is noteworthy that a new therapeutic and relaxation procedure based on the effects of natural shungite ("Shungite chamber") is used in the Czech Republic. The operator company maintains that fullerenes are beneficial in the healing of inflammations and during post-operative recovery, increasing the immunity of the human body.

The first artificial fullerenes were prepared by laser ablation of a graphite target in a helium atmosphere following the method of H. W. Kroto. Fullerenes are marketed, e.g., by *Frontier Carbon Corporation* (FCC) seated in Tokyo, Japan. The process is based on the controlled combustion of organic substances. The outputs are in the order of tons per year, and the potential for substantial production volume increase exists, bringing about cost reductions. Other companies marketing this modification of carbon include:

- *NANO-C*, seated in Massachusetts, USA, manufacturing fullerenes by efficient combustion (see Fig. 2),
- *NeoTechProduct*, seated in St, Petersburg, Russia, manufacturing the product based on a Russian patent and on the ownership of a plasma arc reactor,
- *Marchetti S.r.l.*, seated in Padua, Italy, manufacturing the product from graphite by a technology involving an electric arc.

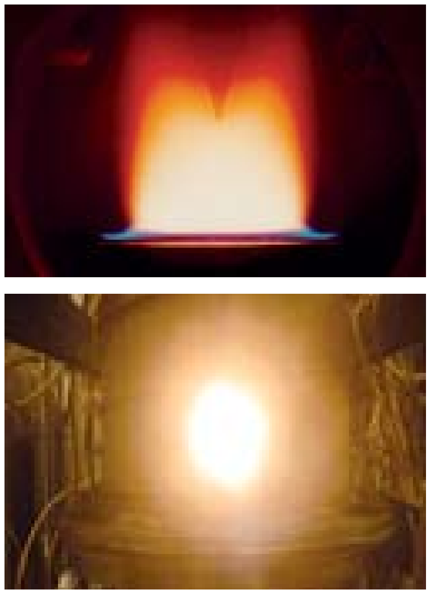

Fig. 2 Fullerene obtained by combustion (Nano-C, 2001)

Reduction combined with addition is employed to improve or obtain the required properties of fullerenes. Reduction was the first chemical conversion of fullerenes, enabling different properties to be imparted to fullerene derivatives. For example, an insulating material can be thus transformed into a superconductor. Addition reactions can give rise to various types of fullerene derivatives, sometimes with important photochemical properties.

Fullerene properties, safety data sheets and uses

A *high hardness* is the most remarkable **property** of fullerenes. The best-known type, hardened fullerene C_{60}, is even superior to diamond in its hardness, thus being the hardest known material worldwide. Its density is only 0.3 % greater than the density of diamond. Young's modulus of elasticity expected for a fullerene crystal based on calculation is 15.9 GPa. *The tensile strength of the nanotubes* can reach a level as high as 63 GPa. *Sound propagation velocity* in fullerene is (2.1 to 4.3) x 10^5 cm.s^{-1} (Girman, 2009).

Chemical reactions of fullerenes are affected by their π-electrons, due to which they react like *aliphatics* rather than aromatics (Lhoták, 2004).

All the C_{60} carbons exhibit sp2 hybridization, their spatial arrangement, however is pyramidal (rather than planar). This brings about a large internal strain and so *C_{60} fullerene is thermodynamically less stable* than graphite (Lhoták, 2004). C_{60} is the thermodynamically most stable fullerene,

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presumably owing to its high symmetry - its shape approaches the sphere most of all among fullerenes. The molecule of fullerene C_{60} is appreciably electronegative, so it is easy to reduce but difficult to oxidize (Girman, 2009). Fullerenes transform into graphite at temperatures above 1 500 °C (Frank et. al., 2010).

Fullerenes doped with alkali metals can be used as superconductors. The highest *superconductivity temperature* is attained with the well-known Cs_3C_{60}. Fullerenes are well soluble in chloronaphthalene (51 $mg.ml^{-1}$) and methylnaphthalene (33 $mg.ml^{-1}$) and not so well soluble in toluene (3 $mg.ml^{-1}$) and benzene (1.5 $mg.ml^{-1}$). They are nearly insoluble (1.3×10^{11} $mg.ml^{-1}$) in water (Girman, 2009).

As follows from the safety data sheets, fullerenes are *irritants* only.

Fullerene nanoparticles have been reported to form an insoluble fullerite with a hydrophobic surface in water (Zemanová and Klouda, 2011). On a water surface, fullerene forms toxic clusters as large as a few centimetres. C_{60} clusters may induce oxidative brain damage in fish. They penetrate into the fish brain, like into the mammalian brain, through the olfactory channel (Zemanová and Klouda, 2011). This information contradicts what is stated in the publication (Girman, 2009): "No *toxic effects* on humans have been identified for fullerenes. No genotoxic or mutagenic potential of fullerenes has been identified either."

Hence, we are faced with the issue of the existence of two contradicting opinions regarding fullerene. Now, the question arises as to whether the benefits of nanomaterials outweigh their adverse impacts on humans and/or on the environment, or not.

The problem of the contradicting opinions may be resolved based on information contained in the safety data sheet, which is the basic document of any hazardous substance or mixture. A safety data sheet must contain identification of the manufacturer or importer, information on the hazardous substance, and information needed to protect human health and the environment. It should enable individuals handling the substance/mixture to take health and environmental protection measures.

Among important data is the CAS number (Tab. 1), assigned by the Chemical Abstracts Service. This classification defines fullerenes as clusters with even numbers of carbon atoms in a molecule, spatially arranged into a spherical or distorted spherical shape (Girman, 2009).

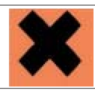
Tab. 1 CAS numbers of some fullerenes

Fullerene C_{60}	99685-96-8
Mixed fullerene C_{60}/C_{70}	131159-39-2
Fullerene C_{70}	115383-22-7
Fullerene C_{76}	135113-15-4
Fullerene C_{78}	136316-32-0
Fullerene C_{84}	135113-16-5

From existing safety data sheets (Tab. 2, Tab. 3) it follows that fullerene is an irritant only. No *toxicological or ecotoxicological* information is available so far. Information on fullerene and its derivatives is insufficient and so we cannot consider one opinion more trustworthy than the other.

Fullerene C_{60} 99.5 %

Tab. 2 Summary information extracted from the SDS of C_{60} (BL C60, 2010)

CAS No.	**99685-96-8**
Classification according to CLP	OKO Irrit. 2, STOT SE 3, H319, H 335.
Pictogram	
H phrases	H319 - Causes serious eye irritation. H335 - May cause respiratory irritation.
P phrases	P261 - Avoid breathing dust/fume/gas/mist/vapours/spray. P280 - Use protective gloves/protective clothes/safety glasses/face shield. P305+P351+P338 - IF IN EYES: Rinse continuously with water for several minutes. Remove contact lenses if present and easy to do. Continue rinsing.
Classification according to DSD - *Dangerous Substances Directive* (Chemical Act)	**Xi, R 36/37**
Symbol	Xi 
R phrases	R 36/37/38 - Irritating to eyes, respiratory system and skin.
S phrases	S26 - In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. S36 - Wear suitable protective clothing.
Physico-chemical properties	
Appearance	powder
Colour	dark brown
Melting point	> 280 °C
Flash point	> 94 °C
Molecular weight	720.64 $g.mol^{-1}$
Density	1.6 $g.cm^{-3}$ at 20 °C
ADR/RID	4.1
Kemler code	40
UN code	1325

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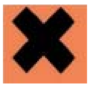
Fullerene C_{70}

Tab. 3 Summary information extracted from the SDS of C_{70} (BL C70, 2010)

CAS No.	**115383-22-7**
Classification according to CLP	OKO Irrit. 2, STOT SE 3, H319, H 335.
Pictogram	
H phrases	H319 - Causes serious eye irritation. H335 - May cause respiratory irritation.
P phrases	P261 - Avoid breathing dust/fume/gas/mist/vapours/spray. P305+P351+P338 - IF IN EYES: Rinse continuously with water for several minutes. Remove contact lens, if present and easy to do. Continue rinsing.
Classification according to DSD - Dangerous Substances Directive (Chemical Act)	**Xi, R 36/37**
Symbol	Xi 
R phrases	R 36/37/38 - Irritating to eyes, respiratory system and skin.
S phrases	S26 - In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. S36 - Wear suitable protective clothing.
Physico-chemical properties	
Appearance	crystals
Melting point	> 280 °C
Molecular weight	840.75 g.mol^{-1}

A safety data sheet has also been developed for bromofullerene $C_{60}Br_{24}$, which is marketed by the MER Corporation seated in Arizona, USA. The manufacturer reports basic information on the compound only, no information on the physico-chemical properties, technical safety parameters or, most importantly, on the health risks is presented (Mer Corporation, 2005).

Manufacturers using fullerenes

The number of manufacturers that use fullerene is greater than one would expect in view of the lack of relevant information. The best-known manufacturers include, e.g., Millers Oils, UNT and YONEX.

Millers Oils - A-SHIFT s.r.o. launched a new generation of high performance gearbox oils (Millers Oils, 2009) for sports and racing applications using nanotechnology (CRX 75w140 NT and CRX LN 75w140 NT) in 2009. The additives are called inorganic fullerenes and are used to supersede additives used so far. When subjected to very high loads, the fullerenes are deformed into small rollers which reinforce the molecules of the oil. As a result, the load capacity of the oil increases, friction is reduced, the oil heats up less, and wear of mechanical parts is alleviated. Tests have demonstrated 25 % lower friction or even better when compared to oils with molybdenum disulfide or PTFE (Millers Oils, 2009).

The manufacturer's safety data sheet does not indicate any serious hazard, only eye and skin irritation/reddening at the site of contact with the material. Nitrile gloves, protective goggles and protective clothing constitute the only PPE recommended, no respiratory protection being required.

As to the environmental hazard, the following combination of R phrases is given: R52/53 Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment. Synthetic oil should be absorbed by soil rapidly and should be biodegradable. The following S phrase is also presented: S2 Keep out of the reach of children.

The skin care company **UNT** (UNT Skincare, 2012) uses fullerene as an antioxidant marketed under the name Radical Sponge® which is supposed to protect skin from the adverse effects of free radicals. The company states on its website that clinical tests have proved the efficiency of Radical Sponge in destroying free radicals on UV exposure. Products containing fullerene are marketed under the name ELIXIRIN C_{60} (Fig. 3).

Fig. 3 Effects of Radical sponge (UNT Skincare, 2012)

YONEX (Technologie Yonex, 2012) has developed a generation of fullerenes with 4 ribs, through which it transforms the fullerene molecules so that the carbon atoms are combined into the structure of a football. X-fullerenes are used in the manufacture of tennis rackets (see Fig. 4) in combination with a resin and carbon fibres, creating a cross-linked structure. X-fullerene increased the racket frame toughness by 6 %, front stability by 25 %, and frame repulsion by 4 %. Thus the racket is more stable during forceful shots and even during off-centre shots.

Fig. 4 Structure of X-fullerenes
(Technologie Yonex, 2012)

Although the danger is sometimes nearly invisible at first glance, the fact that fullerenes may be *toxic* in certain circumstances should be borne in mind. Sometimes this is due to the solvents. Nanoparticles comprise a few atoms, all of them being located near the surface, and so it is easier for them to react with the atoms or molecules of other substances (Klouda and Kubátová, 2010).

Toxicity of fullerene, as mentioned above, is not clear so far. Different attitudes exist. It is clear that fullerenes, like other chemical substances, penetrate into the respiratory or gastrointestinal tract through the skin or by injection. In view of the results of studies devoted to the potential toxicity of fullerene when in contact with skin, stringent precautions should be applied. The use of personal protective equipment when handling fullerenes is imperative (Klouda and Kubátová, 2010). Due to its small size, fullerene can pass through cells in the body and react with them readily.

The behaviour of fullerene on its own and when present in solvents, as well as its interactions with the components of the environment, will presumably depend on the following factors:

- Particle size;
- Particle shape;
- Surface area;
- Solubility;
- Charge;
- Physico-chemical properties.

Carbon nanotubes have been reported to cause pathological changes in animal stomachs similar to those caused by asbestos fibres, and to cause lung inflammation and fibrosis on inhalation. Genotoxic effects of carbon nanotubes have also been observed. Other studies exist, however, where the toxicity of carbon nanotubes does not seem to be that serious. On the contrary, fullerenes appear to be outstanding antioxidants (Prášek, 2011).

Results

Testing the environmental impacts

Environmental impact tests are suitable to gain information on the harmful effect of the substance on plants. Information so gained cannot be obtained by any other way.

The starting fullerene C_{60}, 99.5 % purity, was obtained from SES Research Houston USA and its derivatives for the experiments were prepared at *laboratories under the umbrella of the State Office for Nuclear Safety (SÚJB)*. Apart from the standard test (*Sinapis alba* [white mustard] root growth inhibition) and the phytotest (plant root growth inhibition), the UV radiation test was performed (Drastichová, 2012).

Fullerene bromination through contact with bromine liquid was conducted in carbon tetrachloride, also in the *laboratories under the SÚJB*. The solvent and excess bromine were evaporated and the residue was dried to constant weight. Based on elemental analysis performed by the *Institute of Chemical Technology in Prague (VŠCHT)*, the composition of the product was $C_{60}Br_2$. Additional techniques used to identify the substance included FTIR (Fig. 5 and 6), DTA and TGA (Zemanová and Klouda, 2011; Drastichová, 2012).

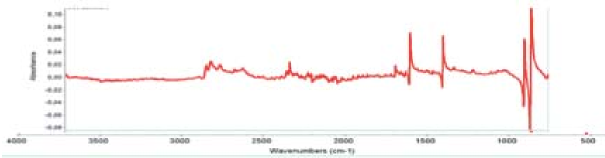


Fig. 5 IR spectrum of the starting fullerene C_{60}

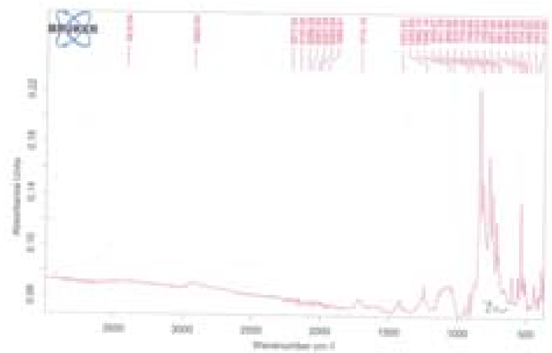


Fig. 6 IR spectrum of the product $C_{60}Br_2$

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Comparison of TGA between the fullerene derivative and the starting fullerene revealed a substantial difference in thermal stability. No decomposition of the starting fullerene was observed up to 487 °C. The first exothermic effect occurred at 540 °C, the next temperature maximum lay at 993 °C. Weight loss of the starting fullerene within the region of 22 °C - 1 100 °C was a mere 56.2 %. The development of thermal decomposition of the fullerene bromo derivative was different (Drastichová, 2012).

The thermal analysis was followed by toxicity testing of the samples using **conventional toxicity tests** and testing of the UV stability of the samples. One test type was represented by the root growth inhibition test on *Sinapis alba*, the other test type was represented by the root growth inhibition test on *Tradescantia fluminensis* (Wandering Jew) and *Peperomia argyreia* applying a concentration of 0.67 $g.l^{-1}$ (Drastichová, 2012).

Fullerene C_{60}

No toxic effect was observed in the first type test, i.e. on the roots of white mustard (Fig. 7). The opposite, i.e. root growth stimulation, was actually observed, although the test duration was extended. While initially the test was planned for 72 hours, the actual test duration was extended by another 7 days of action on the mustard seed (Drastichová, 2012).

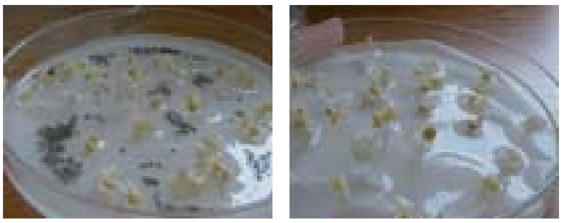

Fig. 7 Seeds in C_{60}, H_2O samples in 72 hours

In the second type test, the effect of fullerene on the growth of the roots and leaves of *Tradescantia fluminensis* was examined (Fig. 8). The results relative to the control sample were unfavourable but not adverse. Four fewer roots grew, two leaves had signs of drying and two leaves had rotted away. Exposure time was 21 days. The adverse effects on the root growth and on the drying of the leaves were most pronounced during the first 8 days. In 16 days, on the contrary, the roots grew faster than in the control sample and 3 roots exceeded the maximum length observed in the control sample. Thus, the test does not enable an unambiguous conclusion to be drawn as regards the toxic effect of fullerene on plants. On the other hand, a conclusion that fullerene was nontoxic cannot be drawn, either (Drastichová, 2012).

Fig. 8 Tradescantia fluminensis in a mixture of C_{60} and 75 ml of water

The UV test (Fig. 9), which was run for 150 hours, did not demonstrate any effect of radiation on fullerene; conversely, it gave evidence of the stability of fullerene under UV radiation (Drastichová, 2012).

Fig. 9 Samples exposed to UV in darkness

Bromofullerene $C_{60}Br_x$

The first type test, on mustard seeds, did not demonstrate any toxic effect of bromofullerene, either (Fig. 10). The roots were stimulated to a greater extent (by roughly 50 %) than with fullerene (Drastichová, 2012).

Fig. 10 Seeds in $C_{60}Br_x$(1), H_2O samples in 72 hours

In the second type test, the adverse effect on the plants was quite appreciable (Fig. 11). The number of roots grown was lower by 4, the leaves showed signs of drying and 2.5 leaves had rotted away. The *Tradescantia* roots were visually weaker than in the control sample, were not branched and their look was reminiscent of thread. The roots did not grow or spread in 8 days. Appreciable growth was only observed in 13 days. The root length did not exceed that in the control sample (Drastichová, 2012).

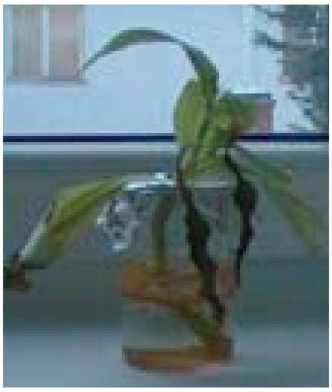

Fig. 11 Tradescantia fluminensis in a mixture of $C_{60}Br_x$ and 75 ml of water

Hence, bromofullerene had an appreciable adverse effect on *Tradescantia fluminensis*, which could be caused by bromine, which is categorized as highly toxic, corrosive and dangerous to the environment (according to the Chemical Act). CLP defines this element as one with acute toxicity cat. 2, causing skin corrosion - cat. 1A, and dangerous to aquatic organisms.

The UV exposure test demonstrated a 7 % to 8 % weight loss against the initial weighed amount. This weight loss may also be due to the bromine in the fullerene molecule: bromine evaporates readily (due to the rather low boiling point, 58 °C) and is unstable (Drastichová, 2012).

Conclusion

In a published experiment where fullerenes had been added to a fish tank, the nanoparticles penetrated through the gills and into the brains of the fish, where they harmed the brain cells. Adverse effects have also been observed in experiments where fullerene was added to a skin cell culture. One-half of cells died readily in a solution containing fullerenes at 20 ppb (Zemanová and Klouda, 2011; Slezák, 2011).

The tests performed by us did not prove the direct toxicity of fullerene or its bromo derivative; the results, however, cannot be regarded as conclusive. The tests should constitute a basis for additional experiments to identify the effects of fullerene and its derivatives on the environment as well as on human health. The high price of fullerene is an unfavourable factor affecting the resources available for experiments. This is why the tests were not adequately repeated, a restricted number of plant specimens was used, and a single fullerene concentration was applied.

If the properties of nanomaterials are unknown, precautionary measures can be effective if based on:

- a list of nanomaterials which are in use, along with their properties where available,
- a description of technological, process and handling operations where nanomaterials are involved,
- a definition of exposure and its limits (technological, organizational, collective or personal protection),
- inspections of workplaces and effectiveness assessment for the measures,
- a description of circumstances where excessive exposure to nanomaterials may occur in emergency situations,
- a list of exposed employees,
- medical examinations of employees who may come into contact with nanomaterials.

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