

THE TOXICITY OF NANOMATERIALS AND ITS MONITORING IN THE LIGHT OF CLASSICAL ENVIRONMENTAL TOXICOLOGY - CHALLENGES AND OPPORTUNITIES

SCHREIBEROVÁ Lenka, HASE Veronika, DANIHELKA Pavel

VSB - Technical University of Ostrava, Czech Republic, EU

Abstract

Nanomaterials are understood as chemicals in the REACH directive and approaches of “classical” toxicology generally applied. Nevertheless, some properties of nanomaterials differ from compounds, e.g. size of basic unit, transport properties, corona etc. Growing use of nanomaterials will lead to their release to the environment and potential, even today unknown behaviour may appear. Human society has wide range of experience with unexpected or unknown toxic effects of engineered compounds (molecules), which may have parallel in nanoscale and which may demand development of new methods. Some examples are: Global pollution by lead in petrol anti-knock. Need of monitoring techniques for NMs in environment distinguishing ENM from natural NM is challenge arising from this parallel. Persistence, long-distance transport and bioaccumulation of POPs show us the necessity to understand and to measure transport properties, behaviour in living organisms an life cycle of ENMs. Mercury transformation to more toxic methylmercury (e.g. Minamata disease) is an example of environmental transformation with increased hazard and its parallel may be transformation of ENM to other, more toxic particles in environment, and even their “invisibility”. Environmental fate studies will need both identification and monitoring technics, only rarely present today. New forms of toxicity and gaps in testing may be recognized in nanoscale, similarly as endocrine disruption or thalidomide effects were unexpected before their discovery. Since the questions about behaviour of nanomaterials in environment will be understood enough, already existing precautionary technics as life cycle analysis and cleaner production may be applied to minimize exposure to nanomaterials throw environment.

Keywords: Engineered nanomaterials, toxicity, persistence, bioaccumulation, transformation

1. INTRODUCTION

In the field of nanotechnology, the extent of the use of engineered nanomaterials (ENMs) is increasing, especially in commercial products, resulting in the expected consequence of increasing the presence of nanoparticles in environmental components and also in the biosphere. Naturally occurring nanoparticles that arise from natural processes (forest fires, volcanic activity, erosions) are also ubiquitous in the environment (E), but nanoparticles of anthropogenic origin (including the targeted production of nanomaterials and other activities) can get into the environment as well, for example as a result of the use of nanopesticides, thermal processes, product abrasion, as emissions at the end of a product's life cycle, etc. Nanomaterials (NMs) existed for a long time before their targeted production began, and organisms exposed to them could adapt to NMs during the course of their evolution. The question therefore arises whether and why to worry and wonder about the impact of NMs on the environment? The reason is our knowledge that even natural NMs may be toxic in some circumstances, for example volcanic dust, and it follows from that similarity that intentionally manufactured nanomaterials (ENMs), which are substances in nanoform that did not occur previously in the environment, may have toxic effects [1]. For each phase of the ENM's life cycle, nanoparticle release can be assumed (either accidentally or intentionally), which leads to the subsequent exposure of human society, and, theoretically, of all components of the environment. Nanoparticles in the biosphere can further react with present elements or molecules, forming new “nanospecific” compounds which may have completely different or new properties compared to the original nanoparticles.

Looking back on history, we can find a wide variety of examples and experiences with the unexpected toxic effects of synthetic chemicals that had not been known until a certain time; these effects led to serious environmental problems, sometimes even global (e.g. DDT and other POPs, methylated mercury). On the basis of the mass use of thoroughly unexplored substances, we can find a certain parallel with nanomaterials because they should be used or applied with some caution until reliable answers to the question of whether NMs pose an increased risk to humans and the environment are found [2, 3, 4, 5, 6, 7, 8].

Human toxicity of fine and ultrafine particles, which can include NMs, is relatively well studied, but the effects on other organisms are largely unknown and the new field of nanoecotoxicology is still in its infancy [1, 4, 9, 10, 11]. The question arises whether NMs, with their unique characteristics, require a special approach and whether the current form of ecotoxicological tests will meet the purposes of testing NMs [1]. The second question is how ecotoxicological laboratory experiments with NMs should be interpreted in ecosystems [1].

2. NANOMATERIALS AS CHEMICAL SUBSTANCES IN THE ENVIRONMENT

The production and application of NMs are no longer solely governed by the laws of classical physics, but also by the rules of molecular and quantum physics; quantum phenomena manifesting at this level lead to entirely new possibilities [12]. As a result, nanomaterials acquire their unique and significantly characteristic properties compared to the same materials when the dimensions of which are larger. Nanomaterials are used e.g. in remedial technologies, in agriculture as a fertilizer, in the food industry, and in many other industries. The potential for their use is far-reaching but, so far, there have been no sufficiently relevant studies that would provide information on whether and to what extent components of the environment are contaminated, which could lead to contamination of the food chain or other exposure of the human population.

One problem nanotechnology has faced on a long-term basis is the still non-existent uniform definition of nanomaterials, which is essential for, inter alia, legislation that regulates their handling. A nanomaterial can be defined as a substance that meets at least one dimension in the nanoscale from 1 to 100 nm. Given that for nanomaterials there are no express requirements under REACH or CLP, the definition is met by substances specified in these regulations, therefore the relevant provisions apply to them [13] and they are considered chemicals.

The behaviour of NMs is very similar to the behaviour of chemicals, and there are a number of physio-chemical processes, such as e.g. aggregation, transformation, dispersion or accumulation. These may also occur in association with NMs released into the environment as a result of their increasing use. According to the authors [3, 4, 6, 10, 14], the opinion prevails that, in most cases, NMs will aggregate after entering the environment, so only a part of them retains the unequivocal nanoform, which will influence the behaviour of these substances in the environment and their impact on the components of ecosystems [1]. The very formation of aggregates is exposed to a number of biotic and abiotic conditions that promote their formation or, conversely, stabilize their dispersion which, consequently, may disrupt already formed aggregates [5, 6, 10]. Moreover, after aggregation of NMs in an aquatic environment, their sedimentation follows, wherein the NMs will be removed from the water column, but they will accumulate at the bottom, thereby becoming potentially hazardous for organisms living near the bottom [1, 5, 7, 15]. The adsorption of the resulting aggregates may occur on the surface and inside organisms [1], which raises the question of the potential bioaccumulation of NMs in aquatic organisms and further along the food chain.

Given that the behaviour of NMs in the environment is a very complex matter and depends on many factors, the question is whether and how NMs will affect other contaminants in the environment [2, 5, 6, 16, 17, 18]. Due to the fact that NMs have a large specific surface area, they can play an important role in the distribution, transport and bioavailability of contaminants in the environment [1, 2, 16, 19]. Remediation technologies based on NMs, which consist of adsorption of contaminants on the NMs, which are specifically dosed into an aquatic or rock environment in order to reduce contamination [16]. A typical example of such an application is cleaning

groundwater contaminated by industrial or technological activities and containing a wide range of persistent organic pollutants (POPs). These waters can be cleaned in-situ by the application of fullerenes, carbon nanotubes or nanoiron [1, 16]. However, the question regarding their influence on toxicity arises when NMs on the one hand can attenuate the toxic effects of POPs, but on the other hand their mobility may be increased [1, 5, 7], which leads to their transportation over great distances. At the same time, according to [1], such mobile NMs can enter the body more easily [5, 7].

3. POSSIBLE PARALLELS BETWEEN CHEMICAL TOXICOLOGY IN THE PAST AND CURRENT NANOMATERIALS

Through human activities chemicals are released into the environment mostly as gaseous, liquid and solid waste, less often than deliberately, e.g. by the use of fertilizers. No substances in the environment stay at rest and their transport and transformations occur in geological and biological processes. E.g., as a result of using nitrate-based fertilizers, their concentrations in surface and ground waters is globally increasing. [20] Let us also recall the effects of chemicals used in the past, such as DDT and heavy metals like lead, cadmium and mercury.

Our experience with “conventional” chemicals, including some unpleasant surprises that humanity has experienced, can be capitalized in the management of nanomaterial safety. Therefore, the following paragraphs describe examples of the toxic effects of chemicals in the past that were unknown for a long time as inspiration for a proactive approach to ensure the safety of nanomaterials.

3.1. A parallel in global contamination - lead

In the past, lead was added in the form of Tetraethyl lead to petrol in order to increase the octane number and hence give higher performance for engines. However, since 1996, Tetraethyl lead has been replaced in petrol by potassium compounds, which are less harmful to the environment [21]. Lead emissions into the atmosphere have the characteristics of long-range transport; they are able to travel great distances and contaminate the components of the environment hundreds of kilometres from the source. After penetrating into the body, lead is stored in bones and, to a lesser extent, in the blood. Interestingly, the authors' studies [22, 23] showed that the ban on leaded fuels to prevent the release of thousands of tons of lead into the atmosphere from cars led to lower levels of lead in the blood of people. Large volumes of nanomaterials which may get into the environment in the future could also lead to global contamination, and similar problems as with lead may arise. For efficient risk management in the case of lead, it was necessary to develop methods for trace analysis and ultra-clean laboratories first, in order to demonstrate global contamination and the fact that it occurred with the development of motoring. For this reason, it is necessary to monitor the possible techniques for NMs in the environment, which would be able to distinguish natural NMs from ENMs. It is also questionable that, in the case of some nanoparticles where specific heteroatoms are not present, a major problem may be identifying nanoparticles in a background of natural materials.

3.2. The parallel of new toxicological properties - bioaccumulable persistent organic pollutants

The oldest and best-known synthetic insecticide used since World War II is DDT, which has gone down in the history of toxicology because of its bioaccumulation ability and persistence. Originally, it was considered very safe because of its low acute toxicity and low doses during application. Likewise, substances of the PCB type were widely used and they were known for their low acute toxicity. Eventually, DDT, PCBs and other substances, now classified as a persistent organic pollutants, were found in the tissues of animals and in locations far distant from the site of their application, which indicates a problem with remote transport. Moreover, their concentrations in organisms were surprisingly high, which was the result of bioaccumulation along the food chain [24, 25]. New, previously unknown forms of toxicity began to manifest as well, such as reproductive toxicity almost leading to extinction of the symbol of the USA - the Bald Eagle - due to reducing

the strength of its eggshells. It therefore appeared that the risk assessment methods used until then did not include new forms of toxicity, and especially new forms of the behaviour of substances in nature (bioaccumulation and persistence). This, coupled with insufficient use of the precautionary principle, led to extensive contamination by POPs across continents. This can imply that new forms of transport processes, accumulation in biological systems and toxicity may also be found in the case of nanomaterials. However, at present, there are not sufficiently selective and sensitive methods for tracking the fate of all types of nanomaterials in the environment and in the human organism, which is a clear challenge for the development of analytical techniques and methods.

It is also important to stress that some pesticides, globally banned by the Stockholm Treaty, are still used in African and Asian countries, e.g. to suppress the occurrence of malaria (DDT, lindane ...). Again, this is an interesting moment in terms of the risk management of nanomaterials; we should not evaluate only their isolated positive or negative effects, but mainly their mutual comparison, and we should perform a parallel of socio-economic analysis required for substances of very high concern (SVHC) under the REACH directives.

Currently, mainly at the laboratory level, the replacement of conventional pesticides by so called nanopesticides is being developed, thanks to which it will be possible to reduce the extent of plant protection product use. Despite the fact that the active substance is encapsulated in the form of nanoparticles, it may get into the soil as a colloid and subsequently contaminate groundwater or surface water. This type of risk to the environment and human health is not yet fully understood; therefore, it is appropriate to identify the behaviour and toxicity of nanopesticides and adequately assess them throughout their life cycle before they are placed on the market. The question arises whether the current testing procedures used for the authorization of plant protection products will be suitable for nanopesticides, or whether specific methods will have to be developed [26].

Another issue to address is the possibility that some nanomaterials will leak into the environment, move therein and bioaccumulate even when they are not directly applied as a pesticide. It may be the case of nanomaterials used e.g. for decontamination, as well as emissions and waste water containing NMs. There is also a clear parallel with toxic chemicals - PCBs, technical materials widely used from the 1920s to 80s, then banned, but still occurring as environmental contaminants.

3.3. Minamata - transformations in the environment leading to increased danger

Minamata is a bay in Japan where another phenomenon characteristic of environmental toxicity was first identified. The gulf waters were contaminated with mercury and in its surroundings an almost epidemic form of a specific disease accompanied by neurological disorders occurred, but it took many years before a causal link between mercury and the disease was found [27]. The problem was that the concentration of mercury in the bay waters and in fish and other products did not correspond to concentrations that would cause any disease, but was much smaller. In addition, the health symptoms were not identical to what could be expected from contamination with inorganic mercury, which was identified in the gulf. The industrial concern Chisso, which was the originator of the contamination, thus refused any association with the disease. Therefore, it took several years before the connection was proven and a few more years before precautionary measures were taken [27]. The problem was that the toxicity was not caused by the initially discharged inorganic mercury (its salt), but by methylmercury produced by its transformation in living organisms; this substance is not only more toxic, but also persistent and bioaccumulable. The new phenomenon was the transformation - toxicity is not caused by the original substance, but by something quite different, which is formed from this substance by natural processes. Chisso was eventually forced to pay compensation totalling more than 250 billion yen.

The parallel to nanomaterials can be their transformation by natural, but also by anthropogenic, processes. Therefore, it is not enough to monitor NM leaks into the environment and the direct exposure of the population, but we must also explore their entire life cycle in nature to the moment of their transfer to a reliably harmless form, and the possible effects of all significant forms of intermediate products or products of changes. The

issue of the necessary development of analytical techniques and toxicological tests for nanomaterials arises again, not only for the original ENMs but also for their products. Not only total transformations may be of particular interest, but also changes in the surface of nanoparticles, such as corona formation or sorption of specific substances altering behaviour in living systems. An example of reactions that could lead to significant changes in their properties is the derivatization of fullerenes by polar substituents, when the originally hydrophobic and thus water-insoluble compounds are transformed into hydrophilic dispersion-forming derivatives.

4. CONCLUSION

In the past, the aforementioned chemicals (lead, DDT, POPs, PCBs or methylated mercury) used on a mass scale, while insufficiently studied, caused a number of environmental problems, which also had a global character. The principle of environmental problems consisted of their previously unknown properties - persistence, bioaccumulation and long-range transport, which was the cause of global contamination. Metabolites of chemicals (DDD and DDE) resulting from DDT, which was used for a long time in the past, can still be found along the food chain in the form of residues. These characteristics may represent a parallel with nanomaterials, which are frequently used in products of everyday use, while the residue can occur long after their application and it can be accumulated.

A very hot topic in the field of nanomaterials is their transformation. The transformation of nanomaterials can be caused not only due to technologies but also due to natural processes, like in the case of mercury and its transformation into methylmercury, which was discovered in the past. For this reason, it is not enough just to track nanomaterial leaks in the environment and the direct exposure of the population to original nanomaterials; it is necessary to study their entire life cycle up to the moment of their transfer to a reliably harmless form. In today's world of monitoring nanoparticles, techniques which would reliably and sufficiently differentiate nanoparticles by type and source when moving in the environment are still missing. Therefore, it is important to focus on the evaluation or monitoring of the sources of contamination, so that we can identify particles or transformed nanoparticles which can cause bioaccumulation or global contamination.

Despite all efforts and initiatives, there are no valid recommendations for safe exposure to nanomaterials; not only for human society, but also for all environmental components. After the experience with the above mentioned chemicals, an effort to preserve the precautionary principle to reduce potential risks is pertinent, because of hitherto insufficient data on the toxicity and ecotoxicity of nanomaterials. The development of new, hitherto missing, analytical techniques or methods and toxicology tests is a challenge for science due to possible parallels between the properties of chemicals and nanomaterials.

ACKNOWLEDGEMENTS

This article was elaborated within the SGC projects „Evaluation of experimental measurement of combustion processes as last life cycle stages with regard to potential risks“ (SP2016/139), LO1403 Innovation for Efficiency and Environment - growth, LD14041 Exposure to nanomaterials, assessment and management of risks in connection with QSAR / QNTR and EU FP7 project NANoREG, grant 310584.

REFERENCES

- [1] SOVOVÁ, Tereza a Vladimír KOČÍ. Ekotoxikologie nanomateriálů. *Chemické listy* [online]. 2012, (106), 82-87 [cit. 2016-09-22]. Dostupné z: http://www.chemicke-listy.cz/docs/full/2012_02_82-87.pdf
- [2] NAVARRO, Enrique, Anders BAUN, Renata BEHRA, et al. Environmental behavior and ecotoxicity of engineered nanoparticles to algae, plants, and fungi. *Ecotoxicology* [online]. 2008, **17**(5), 372-386 [cit. 2016-09-22]. DOI: 10.1007/s10646-008-0214-0. ISSN 0963-9292. Dostupné z: <http://link.springer.com/10.1007/s10646-008-0214-0>

- [3] FRANKLIN, Natasha M., Nicola J. ROGERS, Simon C. APTE, Graeme E. BATLEY, Gerald E. GADD a Philip S. CASEY. Comparative Toxicity of Nanoparticulate ZnO, Bulk ZnO, and ZnCl₂ to a Freshwater Microalga (*Pseudokirchneriella subcapitata*): The Importance of Particle Solubility. *Environmental Science & Technology* [online]. 2007, **41**(24), 8484-8490 [cit. 2016-09-22]. DOI: 10.1021/es071445r. ISSN 0013-936x. Dostupné z: <http://pubs.acs.org/doi/abs/10.1021/es071445r>
- [4] GRIFFITT, Robert J., Jing LUO, Jie GAO, Jean-Claude BONZONGO a David S. BARBER. EFFECTS OF PARTICLE COMPOSITION AND SPECIES ON TOXICITY OF METALLIC NANOMATERIALS IN AQUATIC ORGANISMS. *Environmental Toxicology and Chemistry* [online]. 2008,**27**(9), 1972- [cit. 2016-09-22]. DOI: 10.1897/08-002.1. ISSN 0730-7268. Dostupné z: <http://doi.wiley.com/10.1897/08-002.1>
- [5] BAUN, A., N. B. HARTMANN, K. GRIEGER a K. O. KUSK. Ecotoxicity of engineered nanoparticles to aquatic invertebrates: a brief review and recommendations for future toxicity testing. *Ecotoxicology* [online]. 2008, **17**(5), 387-395 [cit. 2016-09-22]. DOI: 10.1007/s10646-008-0208-y. ISSN 0963-9292. Dostupné z: <http://link.springer.com/10.1007/s10646-008-0208-y>
- [6] HANDY, Richard D., Frank VON DER KAMMER, Jamie R. LEAD, Martin HASSELLÖV, Richard OWEN a Mark CRANE. The ecotoxicology and chemistry of manufactured nanoparticles. *Ecotoxicology* [online]. 2008, **17**(4), 287-314 [cit. 2016-09-22]. DOI: 10.1007/s10646-008-0199-8. ISSN 0963-9292. Dostupné z: <http://link.springer.com/10.1007/s10646-008-0199-8>
- [7] TAO, Xianji, John D. FORTNER, Bo ZHANG, Yiliang HE, Yongsheng CHEN a Joseph B. HUGHES. Effects of aqueous stable fullerene nanocrystals (nC60) on *Daphnia magna*: Evaluation of sub-lethal reproductive responses and accumulation. *Chemosphere* [online]. 2009, **77**(11), 1482-1487 [cit. 2016-09-22]. DOI: 10.1016/j.chemosphere.2009.10.027. ISSN 00456535. Dostupné z: <http://linkinghub.elsevier.com/retrieve/pii/S004565350901220X>
- [8] BLAISE, C., F. GAGNÉ, J.F. FÉRARD a P. EULLAFFROY. Ecotoxicity of selected nano-materials to aquatic organisms. *Environmental Toxicology* [online]. 2008, **23**(5), 591-598 [cit. 2016-09-22]. DOI: 10.1002/tox.20402. ISSN 15204081. Dostupné z: <http://doi.wiley.com/10.1002/tox.20402>
- [9] CRANE, Mark, Richard D. HANDY, John GARROD a Richard OWEN. Ecotoxicity test methods and environmental hazard assessment for engineered nanoparticles. *Ecotoxicology* [online]. 2008, **17**(5), 421-437 [cit. 2016-09-22]. DOI: 10.1007/s10646-008-0215-z. ISSN 0963-9292. Dostupné z: <http://link.springer.com/10.1007/s10646-008-0215-z>
- [10] KLAINE, Stephen J., Pedro J.J. ALVAREZ, Graeme E. BATLEY, et al. NANOMATERIALS IN THE ENVIRONMENT: BEHAVIOR, FATE, BIOAVAILABILITY, AND EFFECTS. *Environmental Toxicology and Chemistry* [online]. 2008, **27**(9), 1825- [cit. 2016-09-22]. DOI: 10.1897/08-090.1. ISSN 0730-7268. Dostupné z: <http://doi.wiley.com/10.1897/08-090.1>
- [11] Nanotechnologie a nanomateriály. *Krajská hygienická stanice Moravskoslezského kraje se sídlem v Ostravě* [online]. Ostrava, 2007 [cit. 2016-09-22]. Dostupné z: http://www.khsova.cz/01_aktuality/nanotechnologie.php?datum=2009-03-18
- [12] Nanomateriály. *ECHA: European chemicals agency* [online]. Helsinki, Finland, 2015 [cit. 2016-09-22]. Dostupné z: <https://echa.europa.eu/cs/regulations/nanomaterials>
- [13] TINKLE, Sally S. NANOTECHNOLOGY: COLLABORATIVE OPPORTUNITIES FOR ECOTOXICOLOGY AND ENVIRONMENTAL HEALTH. *Environmental Toxicology and Chemistry* [online]. 2008,**27**(9), 1823- [cit. 2016-09-22]. DOI: 10.1897/08-266.1. ISSN 0730-7268. Dostupné z: <http://doi.wiley.com/10.1897/08-266.1>
- [14] RAO, C. N. R., A. MÜLLER a A. K. CHEETHAM (eds.). *The chemistry of nanomaterials: synthesis, properties and applications*. Weinheim: Wiley-VCH, c2004. ISBN 3-527-30686-2
- [15] CHRISTIAN, P., F. VON DER KAMMER, M. BAALOUSHA a Th. HOFMANN. Nanoparticles: structure, properties, preparation and behaviour in environmental media. *Ecotoxicology* [online]. 2008,**17**(5), 326-343 [cit. 2016-09-22]. DOI: 10.1007/s10646-008-0213-1. ISSN 0963-9292. Dostupné z: <http://link.springer.com/10.1007/s10646-008-0213-1>
- [16] FARRÉ, Marinella, Krisztina GAJDA-SCHRANTZ, Lina KANTIANI a Damià BARCELÓ. Ecotoxicity and analysis of nanomaterials in the aquatic environment. *Analytical and Bioanalytical Chemistry* [online]. 2009,**393**(1), 81-95 [cit. 2016-09-22]. DOI: 10.1007/s00216-008-2458-1. ISSN 1618-2642. Dostupné z: <http://link.springer.com/10.1007/s00216-008-2458-1>

- [17] BAUN, A., S.N. SØRENSEN, R.F. RASMUSSEN, N.B. HARTMANN a C.B. KOCH. Toxicity and bioaccumulation of xenobiotic organic compounds in the presence of aqueous suspensions of aggregates of nano-C60. *Aquatic Toxicology* [online]. 2008, **86**(3), 379-387 [cit. 2016-09-22]. DOI: 10.1016/j.aquatox.2007.11.019. ISSN 0166445x. Dostupné z: <http://linkinghub.elsevier.com/retrieve/pii/S0166445X07004250>
- [18] ZHANG, Xuezi, Hongwen SUN, Zhiyan ZHANG, Qian NIU, Yongsheng CHEN a John C. CRITTENDEN. Enhanced bioaccumulation of cadmium in carp in the presence of titanium dioxide nanoparticles. *Chemosphere* [online]. 2007, **67**(1), 160-166 [cit. 2016-09-22]. DOI: 10.1016/j.chemosphere.2006.09.003. ISSN 00456535. Dostupné z: <http://linkinghub.elsevier.com/retrieve/pii/S0045653506011805>
- [19] LAPČÍK, Vladimír. *Výrobní a environmentální technologie*. Ostrava: Vysoká škola báňská - Technická univerzita, 2009. ISBN 978-80-248-1960-0.
- [20] Automobile Industry Largest Source of Lead Pollution Today. *Environmental defense fund* [online]. New York, 2016 [cit. 2016-09-22]. Dostupné z: <https://www.edf.org/news/automobile-industry-largest-source-lead-pollution-today>
- [21] SCHNAAS, Lourdes, Stephen J. ROTHENBERG, María-Fernanda FLORES, Sandra MARTÍNEZ, Carmen HERNÁNDEZ, Erica OSORIO a Estela PERRONI. Blood Lead Secular Trend in a Cohort of Children in Mexico City (1987-2002). *Environmental Health Perspectives* [online]. 2004, **112**(10), 1110-1115 [cit. 2016-09-22]. DOI: 10.1289/ehp.6636. ISSN 0091-6765. Dostupné z: <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1247386/>
- [22] PINO, Paulina, Tomás WALTER, Manuel J. OYARZÚN, Matthew J. BURDEN, Betsy LOZOFF, Erica OSORIO a Estela PERRONI. Rapid Drop in Infant Blood Lead Levels during the Transition to Unleaded Gasoline Use in Santiago, Chile. *Environmental Health Perspectives* [online]. 2004, **112**(10), 1110-1115 [cit. 2016-09-22]. DOI: 10.3200/AEOH.59.4.182-187. ISBN 10.3200/AEOH.59.4.182-187. ISSN 0091-6765. Dostupné z: <http://www.tandfonline.com/doi/abs/10.3200/AEOH.59.4.182-187>
- [23] The Story of Silent Spring. *NRDC* [online]. 2015 [cit. 2016-09-22]. Dostupné z: <https://www.nrdc.org/stories/story-silent-spring>
- [24] DDT - A Brief History and Status. *EPA: US Environmental Protection Agency* [online]. 2015 [cit. 2016-09-22]. Dostupné z: <https://www.epa.gov/ingredients-used-pesticide-products/ddt-brief-history-and-status>
- [25] SIDDIQUI, Manzer H., Mohamed H. AL-WHAIBI a Firoz MOHAMMAD. *Nanotechnology and Plant Sciences: Nanoparticles and Their Impact on Plants* [online]. Springer, 2015 [cit. 2016-09-22]. ISBN 978-3-319-14502-0.
- [26] Minamata Disease The History and Measures. *Ministry of Environment: Government of Japan* [online]. Tokyo, Japan [cit. 2016-09-23]. Dostupné z: <http://www.env.go.jp/en/chemi/hs/minamata2002/>
- [27] *Toxicological Effects of Methylmercury* [online]. 1. Washington, DC: National Academies Press, 2000 [cit. 2016-09-23]. ISBN 9780309171717. Dostupné z: <https://books.google.cz/books?id=FNuu6Sm3wHIC&printsec=frontcover&dq=methylmercury&hl=cs&sa=X&ved=0ahUKewjE3Y6pjaXPAhUGOsAKHZ2RDLYQ6AEIJTAB#v=onepage&q=methylmercury&f=false>