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Artificial Sweeteners and the Environment

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Abstract

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Artificial sweeteners (aspartame, saccharin) were tested using the limit concentration (100 mg/l or 100 mg/kg). Model species were duckweed (*Lemna minor*), green algae (*Desmodesmus subspicatus*), mustard seeds (*Sinapis alba*), daphnids (*Daphnia magna*), lettuce seeds (*Lactuca sativa*), and potworm (*Enchytraeus crypticus*). The results indicated that aspartame had statistically negative effects on duckweed and both sweeteners on the reproduction of enchytraeids.

Keywords: aspartame; saccharin; toxicity

Artificial sweeteners belong to additives that occur in many foods and drinks. Their production has been increased due to lower financial costs of their production, processing and use in comparison with beet sugar or other sweeteners of natural origin. Many tons a year have been used for the production and consumption of artificial sweeteners. Nevertheless, they are restricted to testing as chemical substances or mixtures, for the purposes of the REACH legislation (Regulation (EC) No 1907, 2006).

The assessment of their fate in the environment has been studied as well. The water ecosystem is the principal recipient of primary emissions and fate processes taking place in water, including photochemical and biochemical degradation, hydrolysis, partitioning with dissolved and suspended organic matter and settling with particles deposition. The sweeteners have been found in wastewaters, ground waters and surface waters in concentrations up to several tens of µg/l, but consistent with their low K_{OWs} (Perkola & Sainio 2014), no absorption to various types of sediments has been observed (Kirk 2010). For instance, sucralose has been detected in coastal and sea waters. For example, Gan *et al.* (2013a) reported the saccharin presence in surface waters at concentrations up to 0.21 µg/l in China. In a Canadian river watershed, saccaharin at a concentration of 7.2 µg/l was found where both the urban population and the consumption of calorie-reduced beverages were high (Spoeltra *et al*. 2013). Concentrations of saccharin up to 19.7 µg/l were also found in surface waters and up to 137 µg/l in wastewater (Wolf *et al*. 2012). Artificial sweeteners can also be added to animal feed, and leaking from pig manure into ground water has been reported (Perkola & Sainio 2014). The levels of artificial sweeteners have never been publicly described for the Czech aquatic ecosystems. The analytical methods used to determine artificial sweeteners and the levels of these substances in wastewater and surface waters have already been described in a number of articles (Kokotou *et al*. 2012; Gan *et al*. 2013b; Smrčková & Bindzar 2014). However, many artificial sweeteners are not degradable and could also be introduced into the soil environment (Smrčková & Bindzar 2014).

Effects of artificial sweeteners have not yet been studied in depth. In our work, we focused on the screening of aspartame and saccharin effects on various terrestrial or aquatic plants and invertebrates. Neither of these substances is known to occur naturally. They belong to the oldest and the most frequently used artificial sweeteners in human nutrition and that is why they were selected for this study. The concentration of 100 mg/l was selected because it is the highest permissible limit to decide whether the test substance can cause chronic toxicity

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or not according to the REACH regulation (Regulation 1272, Supplement No. 1, 2008).

Saccharin. Saccharin began to be produced in 1878 (Čopíková *et al*. 2013) and it is one of the oldest artificial sweeteners. Saccharin is a common name for the corresponding acid, 1,2-benzoisothiazol-3(2H)-one-1,1-dioxide, its sodium, potassium and calcium salt (a compound in which the group -SO_2-NH- is part of a ring called sultams). In the human body it is excreted unchanged in the urine, it is not metabolised (Čopíková *et al*. 2013) and the unabsorbed portion is excreted with faeces (Velíšek & Hajšlová 2009). Saccharin is very stable, its solutions buffered at pHs ranging from 3.3 to 8.0 were essentially unchanged (Mitchell & Pearson 1991). Saccharin occurs in groundwater due to old landfills, application of fertilizers in agriculture, degradation of sulfonylurea herbicides, irrigation, soil water management, use of sludge as a fertilizer and leaks in the ducts. Saccharin and its salts are found in municipal wastewater and sewage (Velíšek & Hajšlová 2009). It serves not only as a sweetener for human consumption, but also it is registered as an additive for piglets (Switzerland). Smaller amounts of saccharin are used in industry as a galvanic brightener (Velíšek & Hajšlová 2009).

Aspartame. Aspartame is a linear dipeptide methyl ester of L-aspartyl-L-phenylalanine. The stability of aspartame in aqueous media is not entirely satisfactory, especially for certain foods, such as carbonated and still beverages, which are often subjected to many months of storage prior to consumption. As a dipeptide ester, aspartame undergoes both hydrolysis and cyclization reactions. Under acidic conditions, hydrolysis of the ester and amide bonds is favoured, resulting in the formation of its constituent amino acids with a concomitant loss of sweetness. In more neutral and alkaline environments, it cyclises to the corresponding diketopiperazine (Furia 1980). Photodecomposition of aspartame in aqueous solutions under different conditions of light intensity and pH has been studied. Light illumination significantly increased aspartame degradation in an aqueous solution (pH 7), indicating that aspartame was very unstable under the illuminated conditions. In the dark, 91% of aspartame remained in an aqueous solution at pH 7 after 10 h of storage. At 5500 lux of light, however, 39% of aspartame was destroyed in the solution after 10 h of storage. The photodecomposition rate of aspartame varied with the pH of the system. Aspartame degradation was fastest at pH 7.0, followed by pH 4.0 and pH 6.0, in decreasing order (Kim 2010). Available data now suggest that the regular consumption of aspartame is not hazardous (Velíšek & Hajšlová 2009).

MATERIAL AND METHODS

Ecotoxicological methods. Seven ecotoxicological tests were performed in the study. All assays were performed under the controlled test conditions according to the appropriate test guidelines or descriptions:

Freshwater Alga and *Cyanobacteria* – Growth Inhibition Test (OECD 201, 2011).

Daphnia sp. – Acute Immobilisation Test (OECD 202, 2004).

Lemna sp. – Growth Inhibition Test (OECD 221, 2006).

Sinapis alba – Seedling Emergence and Seedling Growth Test (Metodický pokyn 2007).

Terrestrial Plant Test – Seedling Emergence and Seedling Growth Test (OECD 208, 2003).

Enchytraeid Reproduction Test (OECD 220, 2004).

Enchytraeid Avoidance Test (Amorim *et al.* 2008).

Model organisms. The culture of the algae species *Desmodesmus subspicatus* originates from Institute of Botany ASCR, Třeboň, Czech Republic. The daphnids originate from own culture at University of Chemical Technology Prague. *Lemna minor* was to be delivered by the Federal Environmental Agency, Berlin, Germany. The seeds of *Sinapis alba* and *Lactuca sativa* were bought from Oseva Ariva, Ltd., Czech Republic. The enchytraeid culture originates from RECETOX, Masaryk University, Brno, Czech Republic.

Tested concentration. The concentration of 100 mg/l was selected because it is the upper limit to decide whether a substance could cause toxicity or not according to the REACH legislation (Regulation 1272, Supplement No. 1, 2008). Appropriately, the concentration of 100 mg/kg has been used for the soil environment. This study was focused on the comparison of screening for ecotoxicological effects with legislative purposes of the REACH regulation (the chemical compound or mixture is not toxic according this regulation unless the selected EC_{50} value from and ecotoxicological aquatic test with the most sensitive organisms exceeds the level of 100 mg/l). For this reason, only this concentration was tested in the limit test and the used concentrations have not been verified analytically during the experiments.

Test media. In this study, a reference artificial soil was used in the case of soil tests. In soil ecotoxicology, artificial soil of exact composition (10% is dried

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trough peat, 20% fine-grained clay 0.1–1% $CaCO_3$ for pH adjustment and the rest is fine-grained sand) is often used. The media recommended in the relevant legislation were used in water tests. These are the aqueous solutions of salts at such a ratio that the solution fulfil the conditions for optimal growth and survival of the organism (OECD 201, 2011; OECD 202, 2004; OECD 221, 2006; Metodický pokyn 2007).

Test chemicals. In this study was used the sweetener saccharin from the manufacturer F&N dodavatelé Ltd., Tišice, Czech Republic. Each tablet contained sodium bicarbonate, tartaric acid and 16 mg of saccharin. The second sweetener was aspartame from the manufacturer F&N dodavatelé Ltd., Jiřice, Kostelec nad Labem, Czech Republic. Each tablet contained lactose, aspartame (9 mg), acesulfame K (9 mg), sodium bicarbonate and leucine.

Results of analyses. The measured endpoints were calculated by the following equations:

Specific growth rate (duckweed, alga)

$$\mu = (\ln N_t - \ln N_0)/t \quad (1)$$

where: μ – specific growth rate for the control and the limit concentration (100 mg/l); N_t – measured endpoint in time t; N_0 – measured endpoint in time 0; t – time of exposition (h)

Chlorophyll content (duckweed)

$$CH = CH_w/A \quad (2)$$

where: CH_w – whole chlorophyll content (μg); CH – relative chlorophyll content (μg/cm^2); A – size of frond area (cm^2)

Behaviour of enchytraeids

The avoidance endpoint was expressed as the percentage of worms that avoided the treated soil in the test container from the total number of worms in the container. The results were calculated as follows:

$$NR = ((C - T)/N) \times 100 \quad (3)$$

where: C – number of enchytraeids observed in the control soil (individuals); T – number of enchytraeids observed in the test soil (individuals); N – total number of enchytraeids per replicate (individuals)

Inhibition

Growth and survival of test organisms were expressed as inhibition/stimulation (%). The calculation was performed according to the following equation (2):

$$I = ((X_K - X_C)/X_K) \times 100 \quad (4)$$

where: X_K – measured parametr in control; X_C – measured parametr in test concentration; C – number of enchytraeids observed in the control soil (individuals); T – number of enchytraeids observed in the test soil (individuals); N – total number of enchytraeids per replicate (individuals)

Statistical analyses. The arithmetic means and standard deviations were calculated using Excel (Microsoft Inc., Washington, USA). GraphPad Instat, Version 3 (GrapPad Software, Ind., Suite, La Jolla, USA), was used as the program for the statistical evaluation. Data were statistically evaluated by the one-way analysis of variance (ANOVA), using the Tukey-Kramer Multiple Comparisons Test.

Table 1. The mean values of all endpoints for the control, aspartame (A), and saccharin (S)

Species	Endpoint	C. ± SD	A. ± SD	S. ± SD
L. minor	chlorophyll (μg/cm^2)	11.03 ± 2.60	10.48 ± 0.28	17 ± 4**
	specific growth rate:			
	No. of fronds (day^{-1})	0.21 ± 0.12	0.02 ± 0**	0.21 ± 0.12
	frond area (day^{-1})	0.18 ± 0	0.04 ± 0**	0.18 ± 0
S. alba	length of roots (mm/96 h)	45 ± 1.69	40 ± 2.63*	37 ± 2.64*
D. magna	immobilisation (ind./48 h)	0	0	30 ± 0
D. subspicatus	specific growth rate (day^{-1})	1.66 ± 0.21	1.60 ± 0.91	1.61 ± 0.90
L. sativa	length of roots (mm/5 days)	16 ± 6	17 ± 7	17 ± 6
E. crypticus	surviving (ind./28 days)	10 ± 0	10 ± 0	10 ± 0
	No. of juveniles (ind./28 days)	103 ± 2	46 ± 26**	57 ± 17**
	avoidance behaviour (48 h)	–	16 ± 0	32 ± 0

The concentration of sweeteners is 100 mg/l (aquatic tests) or 100 mg/kg (soil tests); data were statistically assessed using the Tukey-Kramer Multiple Comparisons Test for a statistically significant level of α = 0.05* and α = 0.01**; C – control; A – aspartame; S – saccharin; SD – standard deviation

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Table 2. Effects of artificial sweeteners: inhibition of the measured parameters (%) in comparison with the control

Sweetener	*L. minor*			*S. alba*	*D. magna*	*D. subspicatus*	*L. sativa*	*E. crypticus*
	Ch	area	fronds					
Aspartame	5	79	91	–13	0	4	–6	56
Saccharin	–56	2	3	8	30	3	–6	45

Ch – chlorophyll concentration, area – frond area, fronds – number of fronds; *S. alba* – root growth; *D. magna* – immobilisation of individuals; *D. subspicatus* – specific growth rate; *L. sativa* – root growth; *E. crypticus* – reproduction

RESULTS AND DISCUSSION

The results suggest that aspartame was toxic to duckweed and enchytraeids (Tables 1 and 2). In duckweed there was a negative impact on the number of duckweed fronds and their growth, which could have been caused by biochemical changes, which have an impact on the overall condition of plants (Van Stempvoort *et al.* 2011). In the case of enchytraeids, the obtained results cannot be compared with the data from the literature, because studies describing the effects of artificial sweeteners in soils do not exist. In any case, it is interesting that enchytraeids did not escape from the contaminated soil (Kobetičová & Fryčová 2014). However, when enchytraeids occurred in the contaminated soil, and there was no escape into the clean control soil, the production of juveniles decreased approximately by one half (Tables 1 and 2). A similar effect was observed when saccharin tablets were tested (Tables 1 and 2). We therefore assumed that enchytraeids may not have chemoreceptors to detect saccharin and aspartame in their surroundings, and therefore they cannot escape or die. But the exposure to artificial sweeteners in the soil can lead to damage to their physiological processes manifested by the production of a smaller number of cocoons with eggs. It was found that aspartame at pH higher than 5 and at normal room temperature (at which the underlying tests took place in the thermostat) can degrade to diketopiperazine (Smrčková & Bindzar 2014). Aspartame had no negative effect on the other test organisms (Tables 1 and 2). Unlike aspartame saccharin had no negative effects on duckweed. Conversely, the production of chlorophyll increased. But it is questionable whether the outcome is in a positive sense or not. Stimulation of the monitored parameter does not always mean only an advantage for the test organism. In this case, it could be a certain plant response to stress conditions because the increased content of photosynthetic pigments was not accompanied by the faster growth of plants. Enhancing photosynthesis there could be related to the maintenance of the necessary metabolic activity of affected plants, e.g. the production of antioxidant enzymes (Horemans *et al*. 2014). In addition to the above-mentioned enchytraeids, saccharin had partly an effect on *Daphnia* immobilisation (30%). But unlike aspartame, saccharin does not degrade. We can therefore assume that if an effect of saccharin was found in pill testing, it might be due to the presence of this substance. The results showed that neither of sweeteners affected the robust type of parameters such as survival of daphnia and enchytraeids or prolongation of the plant roots or algal biomass. Negative effects were observed in the more sensitive parameters, such as metabolism of plants (duckweed) and enchytraeid reproduction.

CONCLUSIONS

In this study, artificial sweeteners were tested using only the limit concentration (100 mg/l or 100 mg/kg) and our results indicated the biological toxic effects. Aspartame was more toxic than saccharin in the limit tests. The duckweed was the most sensitive aquatic organism and enchytraeids were the most sensitive organisms in soil. According to the regular legislation (Regulation 1272, Supplement No. 1, 2008), aspartame should be classified into one of the classification classes of hazards to the aquatic environment. No statistically significant adverse effect upon short-term exposure was identified but both the artificial sweeteners caused a negative effect after prolonged exposure (*Lemna minor*, *Enchytraeus crypticus*). It would therefore be certainly interesting to study effects of artificial sweeteners mainly in relation to the biochemical response in plants and soil invertebrates.

References

Amorim M.J.B., Novais S., Römbke J., Soares A.M.V.M. (2008): Avoidance test with *Enchytraeus albidus* (En-

doi: 10.17221/220/2015-CJFS

chytraeidae): Effects of different exposure time and soil properties. Environmental Pollution, 155: 112–116.

Čopíková J., Moravcová J., Wimmer Z., Opletal L., Lapčík O., Dražar P. (2013): Náhradní sladidla. Chemické listy, 107: 867–874.

Furia T. E. (1980): CRC Handbook of Food Additives. 2nd Ed. Boca Raton, CRC Press: 197.

Gan Z., Sun H., Feng B., Wang R., Zhang Y. (2013a): Occurrence of seven artificial sweeteners in the aquatic environment and precipitation of Tianjin, China. Water Research, 47: 4928–4937.

Gan Z., Sun H., Wang R., Feng B. (2013b): A novel solid-phase extraction for the concentration of sweeteners in water and analysis by ion-pair liquid chromatography triple quadrupole mass spectrometry. Journal of Chromatography A, 1274: 87–96.

Horemans N., Van Hess M., Van Hoeck A., Saenen E., De Meutter T., Nauts R., Blust R., Vandenhove H. (2015): Uranium and cadmium provoke different oxidative stress responses in *Lemna minor* L. Plant Biology, 17 (Suppl. 1): 91–100.

Kirk A.M. (2010): Photochemical fate of the artificial sweetener sucralose in the environment. [Thesis.] University of North Carolina Wilmington in Partial Fulfillment of the Requirements for the Degree of Department of Chemistry and Biochemistry, University of North Carolina Wilmington.

Kobetičová K., Fryčová Z. (2014): Jak kroužkovcům chutnají umělá sladidla? In: Pouzar M. (ed.): Průmyslová toxikologie a ekotoxikologie, May 5–7, 2014, Kouty nad Desnou, Univerzita Pardubice and VUOS, s.r.o., Czech Republic.

Kokotou M.G., Asimakopoulos A.G., Thomaidis N. (2012): Artificial sweeteners as emerging pollutants in the environment: analytical methodologies and environmental impact. Analytical Methods, 4: 3057–3070.

MŽP ČR (2007): Metodický pokyn odboru odpadů ke stanovení ekotoxicity odpadů. Prague.

OECD 201 (2011): Alga, growth inhibion test. Guidelines for the Testing of Chemicals No. 201 Organization for Economic Cooperation and Development, Paris.

OECD 202 (2004): *Daphnia* s.p. acute immobilisation test. Guidelines for the Testing of Chemicals No. 202. Organization for Economic Cooperation and Development, Paris.

OECD 208 (2003): Terrestrial plants, growth test. Guidelines for the Testing of Chemicals, No. 208. Organization for Economic Cooperation and Development, Paris.

OECD 220 (2004): Enchytraeid reproduction test. Guidelines for the Testing of Chemicals, No. 220. Organization for Economic Cooperation and Development, Paris.

OECD 221 (2006): *Lemna* sp. growth inhibition test. Guidelines for the Testing of Chemicals, No. 221. Organization for Economic Cooperation and Development, Paris.

Perkola N., Sainio P. (2014): Quantification of four artificial sweeteners in Finnish surface waters with isotope-dilution mass spectrometry. Environmental Pollution, 184: 391–396.

Regulation (EC) No. 1272/2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006, Supplement No. 1: 353.

Regulation (EC) No 1907/2006 – REACH – the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) and establishing a European Chemicals Agency.

Renwick A.G., Thompson J.P., O'Shaughnessy M., Walter E.J. (2004): The metabolism of cyclamate to cyclohexylamine in humans during long-term administration. Toxicology and Applicated Pharmacology, 196: 367–380.

Smrčková Š., Bindzar J. (2014): Náhradní sladidla jako poputanty vody. Chemické listy, 108: 1125–1132.

Spoelstra J., Schiff S.L., Brown S.J. (2013): Artificial sweeteners in a largecanadian river reflect human consumption in the watershed. PLoS ONE 8(12): e82706. doi: 10.1371 /journal.pone.0082706.

Van Stempvoort D.R, Roy J.W., Brown S.J., Bickerton G. (2011): Artificial sweeteners as potential tracers in groundwater in urban environments. Journal of Hydrology, 401: 126–133.

Velíšek J., Hajšlová J. (2009): Chemie potravin 2. OSSIS: Havlíčkův Brod.

Wolf L., Zwiener C., Zemann M. (2012): Tracking artificial sweeteners and pharmaceuticals introduced into urban groundwater by leaking sewer networks. Science of Total Environment, 430: 8–19.

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