Effect of allyl isothiocyanate on developmental toxicity in exposed *Xenopus laevis* embryos

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ABSTRACT

The pungent natural compound allyl isothiocyanate isolated from the seeds of *Cruciferous* (*Brassica*) plants such as mustard is reported to exhibit numerous beneficial health-promoting antimicrobial, antifungal, anticarcinogenic, cardioprotective, and neuroprotective properties. Because it is also reported to damage DNA and is toxic to aquatic organisms, the objective of the present study was to determine whether it possesses teratogenic properties. The frog embryo teratogenesis assay-*Xenopus* (FETAX) was used to determine the following measures of developmental toxicity of the allyl isothiocyanate: (a) 96-h LC50, defined as the median concentration causing 50% embryo lethality; (b) 96-h EC50, defined as the median concentration causing 50% malformations of the surviving embryos; and (c) teratogenic malformation index (TI), equal to 96-h LC50/96-h EC50. The quantitative results and the photographs of embryos before and after exposure suggest that allyl isothiocyanate seems to exhibit moderate teratogenic properties. The results also indicate differences in the toxicity of allyl isothiocyanate toward exposed embryos observed in the present study compared to reported adverse effects of allyl isothiocyanate in fish, rodents, and humans. The significance of the results for food safety and possible approaches to protect against adverse effects of allyl isothiocyanate are discussed.

1. Introduction

Allyl isothiocyanate is a pungent and volatile, colorless oil that is synthesized in *Brassica* plants (mustard, horseradish, radish, wasabi) of the *Cruciferae* family. After breakage of the seeds of this group of plants, the glucosinolate (sinigrin) undergoes glucosinolase (myrosinase)-catalyzed hydrolysis to produce allyl isothiocyanate (Fig. 1) and other compounds [2,3].
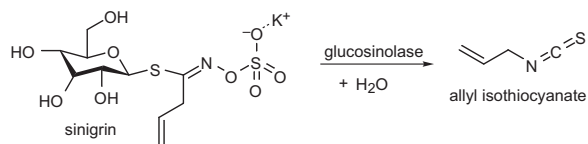
Interest in allyl isothiocyanate arises from the fact that it is reported to exhibit numerous health-promoting beneficial effects. These include (a) antifungal activities in bread [4] and on corn kernels and corn flour [5]; (b) antimicrobial effects against multiple foodborne pathogens and spoilage bacteria in laboratory media, water, and on/in food [4,6–21]; (c) nematicidal effects against *Meloidogyne incognita* [22]; (d) inhibition of mycotoxin toxicity [23]; (e) insecticidal activity against scarab beetle larvae (Coleoptera: Scarabedaes), contaminants of ornamental crops [24]; (f) trypanocidal activity against *Trypanosoma brucei* parasites [25]; and (g) anticancer, cardioprotective,

Fig. 1. Enzymatic hydrolysis of allyl glucosinolate (sinigrin) by glucosinolase (myrosinase) to allyl isothiocyanate [1].

neuroprotective, antidiabetic, and other beneficial properties [26–29]. It is also relevant to note that SH-containing amino acids and peptides such as cysteine and glutathione that are present in meat and in vivo interfered with the antimicrobial activity allyl isothiocyanate against *Escherichia coil* O157:H7, presumably by forming inactive adducts [30,31].

Because it has been reported that allyl isothiocyanate is highly bioactive in cells, animals, and humans, it was also investigated for possible toxicity. Allyl isothiocyanate is reported to induce hyperalgesia and edema in rats [32] and both DNA damage and repair in humans [33] and has been studied for use as a biofumigant due to its irritant properties [34,35]; it is therefore of interest to study the effect of allyl isothiocyanate exposure in humans, particularly in environmental and dietary settings. Adverse effects in humans include low-grade allergy and altered drug metabolism [36].

A report by the European Panel on Food Additives [37] that is relevant to the present study states the following: "The Panel considered that allyl isothiocyanate did not show any evidence of developmental toxicity in pregnant rats, hamsters and rabbits at oral doses of 18.5 mg/kg bw/day, 23.8 mg/kg bw/day and 12.3 mg/kg bw/day, respectively. Allyl isothiocyanate may be fetotoxic to mice at doses higher than 6.0 mg/kg bw/day, without exhibiting any teratogenic effects. The Panel noted that no reproductive toxicity study (one or two generation) was provided by the petitioner and that no information was identified in the published literature."

Allyl isothiocyanate has been shown to possess genotoxic properties in rats that result in the formation of papillomas and hyperplasia [35,38]. Reported 50% lethal doses (LD50) for rats ($\geq$200 mg/kg body weight) and mice ($\geq$50 mg/kg body weight) [39,40] suggest relative low toxicity in rodents. Because a lower lethal concentration in 50% of the population (LC50) for *Pimephales promelas* (fathead minnow) of 85.6 μg/L (0.863 μmol/L) [41] implies that aquatic species might be susceptible to possible adverse effects, the objective of the present study was to find out if this highly reactive molecule has the potential to induce teratogenicity in frog embryos. To the best of our knowledge, this is the first report on teratogenicity of allyl isothiocyanate.

2. Materials and methods

2.1. Materials

Allyl isothiocyanate (cat. No. AAA02901-18) was obtained from Aldrich–Sigma (St. Louis, MO, USA). The following chemicals were obtained from Sigma (St. Louis, MO, USA): L-Cysteine (cat. No. C7352); human chorionic gonadotropin (cat. No. CG5); MS-222 (cat. No. E10521); calcium sulfate (cat. No. C-3771); magnesium sulfate (cat. No. M-7506); sodium bicarbonate (cat. No. S8875); potassium chloride (cat. No. P-5405); calcium chloride (cat. No. C-1016); sodium chloride (cat. No. S7653).

2.2. Animal care and husbandry

Xenopus frogs were purchased from Xenopus I, Inc. (Dexter, MI, USA) and housed in a glass aquaria recirculation system with two to four frogs per 10 gallons of dechlorinated tap water. Human chorionic gonadotropin (250–500 IU) was injected in the dorsal lymph sac of both male and female frogs to induce breeding. Breeding pairs were placed in false-bottom breeding chambers and embryos were collected the next morning. The jelly coat was removed by swirling the embryos for 1–3 min in a 2% L-cysteine solution (pH 8.1). Embryos were then rinsed and placed into sorting dishes. Embryos were double sorted into test dishes of 20 embryos per 8 mL of FETAX test solution in plastic disposable 60 mm × 15 mm Petri dishes. The negative control was FETAX solution (0.625 g/L NaCl, 0.086 g/L NaHCO$_3$, 0.03 g/L KCl, 0.015 g/L CaCl$_2$, 0.006 g/L CaSO$_4$·2H$_2$O, and 0.075 g/L MgSO$_4$ in distilled ASTM type I water) [42]. Frogs were not bred more than once every 2 months.

2.3. FETAX assay procedures

FETAX was used to determine allyl isothiocyanate toxicity and teratogenesis according to ASTM International Guide for FETAX and our earlier studies with teratogenic glycoalkaloids [42–50] and acrylamide [51]. Stock solutions of the test compound were made in FETAX solution. Appropriate dilutions were made to achieve final concentrations. Each day of the four-day test, new solutions were placed into 60 mm covered glass Petri dishes with various concentrations of test compounds dissolved in FETAX solution. At 24, 48, and 72 h, all solutions were prepared and replaced in the dishes. Dead embryos were removed, and live embryos counted. The embryos were cultured at 24 °C. At 96-h, surviving embryos were photographed for length measurements and verified that >90% of controls had reached stage 46. Stage 46 embryos possess hind-limb buds and tightly coiled guts but do not yet feed. At the end of 96-h, the mortality was determined and embryos were anesthetized with MS-222. Malformed survivors, dead embryos, and the developmental stage were observed under a dissecting microscope and recorded according to the Atlas of Malformations [52].

Malformations were determined as follows. All embryos were observed under a dissecting microscope for malformed survivors, developmental stage, and dead embryos. *Xenopus laevis* embryos are transparent and allow for easy determination of gut, heart, head, axial and other internal and external malformations. The malformations are quantitatively scored on a standard score sheet and tallied. The data were recorded according to the ASTM International Guide [43]. The total number of abnormal tadpoles divided by the total number of living tadpoles multiplied by 100 is

the percentage malformed from each dish. The teratogenic index (TI = 96-h LC50/96-h EC50) was calculated from the experimental data.

For length measurements, embryos were photographed with an Olympus stylus 720sw (Olympus Corp. America, Center Valley, PA, USA) on macro setting and analyzed using Image Pro Plus. Detailed photographs were taken of selected embryos using a Pro Reg digital camera (Media Cybernetics, Rockville, MD, USA) attached to a Nikon dissection microscope (Nikon Instruments, Melville, NY, USA). As a measure of growth, head-tail length was measured by the following body contour using a computer equipped with digitizing software.

2.4. Allyl isothiocyanate toxicity experimental design and analysis

For concentration-response studies of toxic effects on embryos, four negative controls (four replicates) and nine test concentrations (two replicates each) of allyl isothiocyanate ranging from 1 to 5 µM were tested increasing in geometric progression. The experiment was repeated three times. Each experiment used 440 embryos. The 96-h LC50 (concentration causing 50% lethality), 96-h EC50 (concentration inducing malformation or gross terata in 50% of surviving embryos) was calculated for each experiment and the teratogenic index (TI) was determined as the 96-h LC50/96-h EC50 (malformation).

2.5. Statistics

Probit analysis was used to determine the 96-h LC50 and 96-h EC50 values using Systat 13 (Systat Software, Inc., Chicago, IL, USA). ANOVA was used to determine if treatments were significantly different ($p \leq 0.05$) from each other using the Bonferroni Post Hoc Test for mortality, malformation, and embryo length. This was used to determine the No-Observed-Effect Concentration (NOEC) and Lowest-Observed Effect Concentration (LOEC) for mortality and malformation. The Minimum Concentration to Inhibit Growth (MCIG) was also calculated using the Bonferroni post hoc test.

3. Results and discussion

The natural compound allyl isothiocyanate is known to be a pungent irritant [34]. The dermal blistering and gastrointestinal irritation of *Xenopus* embryos (Fig. 2) in this study fits this profile of a caustic agent, though the isolated compound is notably more purified and potentially more potent than in mustard extracts. In nature, allyl isothiocyanate comes along with other compounds present in the natural source, whereas this experiment used the purified oil. This might exacerbate some of the malformations seen here compared with those seen with the naturally occurring mixture.

Fig. 2 illustrates the common malformations seen with allyl isothiocyanate under the test conditions include blistering, edema (dermal swelling), and deterioration of the gut. Fig. 3 shows the concentration response from a representative experiment (#2). The curves in the diagram
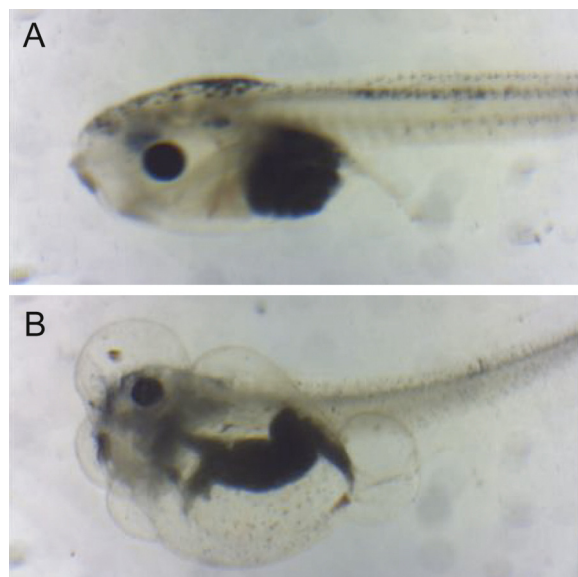

Fig. 2. Photomicrographs comparing normal *Xenopus* embryo (A) with malformed *Xenopus* embryo after exposure to 4 µM allyl isothiocyanate (B).

indicate quite a separation between mortality and malformation. The malformation curve has a sharper slope and may indicate that there is a different mode of action for the compound at concentrations that cause malformation than those that cause mortality. Three separate experiments were performed and results indicate very repeatable results for allyl isothiocyanate (Table 1).

From this study, the LC50 lethality data for allyl isothiocyanate in *Xenopus* was found to be average 4.69 µmol/L (Table 1), which is about five times less toxic than in fathead minnows. The average 96-h EC50 malformation was 2.98 µmol/L (Table 1). These numbers give an average
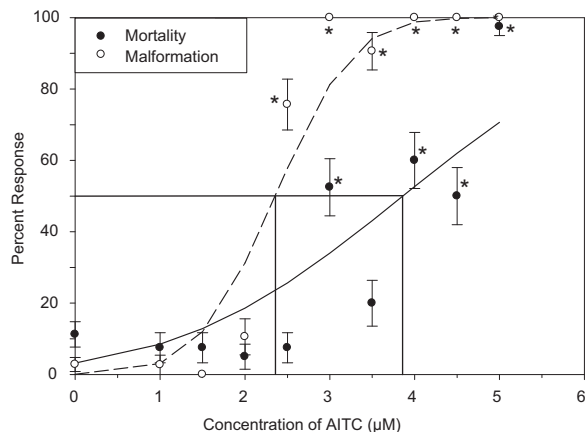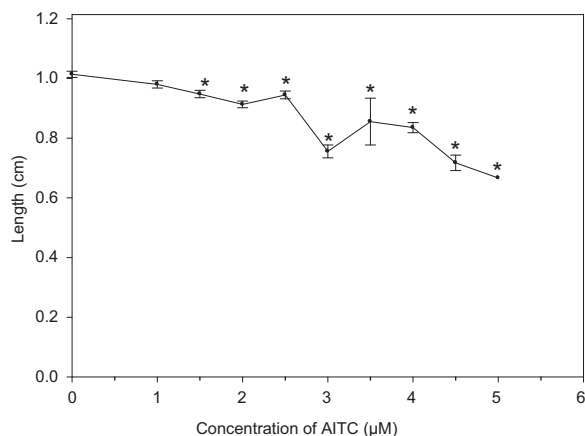

Fig. 3. Concentration-response mortality and malformation data for a representative trial as a function of allyl isothiocyanate concentration. * indicates significant difference from controls at a $p < 0.05$. Error bars are standard error. The vertical lines indicate estimates of the 96-h LC50 and 96-h EC50.

Table 1
The median lethal concentration (96-h LC50), median effective concentration (96-h EC50), teratogenic index (TI = 96-h LC50/96-h EC50), no-observed-effect-concentration (NOEC), lowest-observed-effect-concentration (LOEC) for mortality and malformation, and minimum concentration to inhibit growth (MCIG) for allyl isothiocyanate in FETAX.

Experiment	Total embryos	96-h LC50 lethality[a]	96-h EC50 malformation[a]	TI	Mortality[a]		Malformation[a]		MCIG growth inhibition[a]
					NOEC	LOEC	NOEC	LOEC	
1	440	3.84 (3.42–4.45)	2.63 (2.45–2.86)	1.5	3.0	3.5	2.5	3.0	2.0
2	440	3.86 (3.55–4.24)	2.35 (2.20–2.52)	1.6	3.5	4.0	2.0	2.5	1.5
3	440	6.38 (5.52–8.03)	3.95 (3.63–4.38)	1.6	4.5	5.0	2.0	2.5	5.0
Average		4.69	2.98	1.56	3.66	4.16	2.16	2.66	2.83

[a] All concentrations are in μM. The 95% Fieller bounds are shown in parenthesis.

Fig. 4. Decreasing lengths of embryos from a representative trial as a function of allyl isothiocyanate concentration. * indicates significant difference from controls at a $p < 0.05$. Error bars are standard error.

teratogenic index (TI) value of about 1.6. Because the TI value is >1.5, this suggest possible moderate teratogenicity.

Fig. 4 shows that the average length of the embryos tended to decrease with increasing concentration of allyl isothiocyanate causing significant decrease in embryo length at approximately 60% of the 96-h LC50 values or 2.83 $\mu mol/L$. The TI value combined with severity of the malformations and growth impacts seen indicate that allyl isothiocyanate is a potential teratogen.

It is instructive to compare the quantitative data from reported previous studies with fathead minnows and the results of the present study with the *Xenopus* embryos. There are several possible reasons for the difference in the LC50 value observed between fathead minnows and *Xenopus* embryos. First, the fathead minnow studies were performed using a flow-through bioassay, whereas FETAX uses a concentration renewal procedure. This would affect the relative exposure rates of each species. Next, fathead minnow toxicological studies can be performed with larval or adult specimens, which can alter the LC50 values dramatically. FETAX, on the other hand, is specifically designed to study embryos. This leads into the third point. In FETAX, blastula through Stage 46 larvae embryos are used, and dermal permeability to water is potentially higher than in larvae *Xenopus* or scaled fishes due to the thinner dermal tissue and direct uptake of water through gills. Also, allyl isothiocyanate is a somewhat water soluble oil so there is the potential for passage through the lipid layers.

Therefore, although the difference between the species is notable, it is within a reasonable comparative range.

From this data, it seems that allyl isothiocyanate might be more harmful in the first stages of human life, but humans may have developed better systems to metabolize it. It is not known to what extent, if any, the adverse effects of allyl isothiocyanate observed in the present study may impact its use as a health-promoting compound mentioned in the Introduction, especially as an antimicrobial food additive.

It also appears that the reported toxicity of allyl isothiocyanate against microbial, fungal, trypanosomal, animal, and human cells parallels the activity of this highly bioactive natural compound against the frog embryos. We are challenged to find out whether SH-containing amino acids and peptides such as ʟ-cysteine, N-acetyl-ʟ-cysteine, and reduced glutathione which, as mentioned earlier, protected the foodborne *Escherichia coli* bacteria against inactivation by allyl isothiocyanate as well as against acrylamide-induced teratogenicity in the frog embryos, would also protect allyl isothiocyanate against teratogenicity observed in the present study.

Conflict of interest

The authors declare that there are no conflicts of interest.

Transparency document

The Transparency document associated with this article can be found in the online version.

Acknowledgments

References

[1] R.C. Lindsay, Flavors, in: S. Damodaran, K.L. Parkin, O.R. Fennema (Eds.), Fennema's Food Chemistry, fourth ed., CRC Press/Taylor & Francis, Boca Raton, FL, 2008, pp. 639–687.

[2] R. Dai, L.-T. Lim, Release of allyl isothiocyanate from mustard seed meal powder, J. Food Sci. 79 (2013) E47–E53, http://dx.doi.org/10.1111/1750-3841.12322.

[3] H.K. Sharma, S. Ingle, C. Singh, B.C. Sarkar, A. Upadhyay, Effect of various process treatment conditions on the allyl isothiocyanate extraction rate from mustard meal, J. Food Sci. Technol. 49 (2012) 368–372, http://dx.doi.org/10.1007/s13197-011-0282-7.

[4] I. Azaiez, G. Meca, L. Manyes, M. Fernández-Franzón, Antifungal activity of gaseous allyl, benzyl and phenyl isothiocyanate in vitro and their use for fumonisins reduction in bread, Food Control 32 (2013) 428–434, http://dx.doi.org/10.1016/j.foodcont.2013.01.020.

[5] I. Azaiez, G. Meca, L. Manyes, F.B. Luciano, M. Fernández-Franzón, Study of the chemical reduction of the fumonisins toxicity using allyl, benzyl and phenyl isothiocyanate in model solution and in food products, Toxicon 63 (2013) 137–146, http://dx.doi.org/10.1016/j.toxicon.2012.12.010.

[6] A.C. Chan, D. Ager, I.P. Thompson, Resolving the mechanism of bacterial inhibition by plant secondary metabolites employing a combination of whole-cell biosensors, J. Microbiol. Methods 93 (2013) 209–217, http://dx.doi.org/10.1016/j.mimet.2013.03.021.

[7] W. Chen, T.Z. Jin, J.B. Gurtler, D.J. Geveke, X. Fan, Inactivation of Salmonella on whole cantaloupe by application of an antimicrobial coating containing chitosan and allyl isothiocyanate, Int. J. Food Microbiol. 155 (2012) 165–170, http://dx.doi.org/10.1016/j.ijfoodmicro.2012.02.001.

[8] J.R.D. David, A. Ekanayake, I. Singh, B. Farina, M. Meyer, Effect of white mustard essential oil on inoculated Salmonella sp. in a sauce with particulates, J. Food Prot. 76 (2013) 580–587, http://dx.doi.org/10.4315/0362-028X.JFP-12-375.

[9] M.V. Dias, F. De Fátima, N. Soares, S.V. Borges, M.M. De Sousa, C.A. Nunes, I.R.N. De Oliveira, E.A.A. Medeiros, Use of allyl isothiocyanate and carbon nanotubes in an antimicrobial film to package shredded, cooked chicken meat, Food Chem. 141 (2013) 3160–3166, http://dx.doi.org/10.1016/j.foodchem.2013.05.148.

[10] M. Guo, T.Z. Jin, O.J. Scullen, C.H. Sommers, Effects of antimicrobial coatings and cryogenic freezing on survival and growth of Listeria innocua on frozen ready-to-eat shrimp during thawing, J. Food Sci. 78 (2013) M1195–M1200, http://dx.doi.org/10.1111/1750-3841.12180.

[11] T. Jin, J.B. Gurtler, Inactivation of Salmonella in liquid egg albumen by antimicrobial bottle coatings infused with allyl isothiocyanate, nisin and zinc oxide nanoparticles, J. Appl. Microbiol. 110 (2011) 704–712, http://dx.doi.org/10.1111/j.1365-2672.2011.04938.x.

[12] T.Z. Jin, J.B. Gurtler, S.-Q. Li, Development of antimicrobial coatings for improving the microbiological safety and quality of shell eggs, J. Food Prot. 76 (2013) 779–785, http://dx.doi.org/10.4315/0362-028X.JFP-12-460.

[13] F. Mushantaf, J. Blyth, M.R. Templeton, The bactericidal effects of allyl isothiocyanate in water, Environ. Technol. 33 (2012) 2461–2465, http://dx.doi.org/10.1080/09593330.2012.671855.

[14] A.N. Olaimat, R.A. Holley, Effects of changes in pH and temperature on the inhibition of Salmonella and Listeria monocytogenes by allyl isothiocyanate, Food Control 34 (2013) 414–419, http://dx.doi.org/10.1016/j.foodcont.2013.05.014.

[15] Y.-H. Pang, S. Sheen, S. Zhou, L. Liu, K.L. Yam, Antimicrobial effects of allyl isothiocyanate and modified atmosphere on Pseduomonas aeruginosa in fresh catfish fillet under abuse temperatures, J. Food Sci. 78 (2013) M555–M559, http://dx.doi.org/10.1111/1750-3841.12065.

[16] I.M. Pérez-Díaz, R.F. McFeeters, Preservation of acidified cucumbers with a natural preservative combination of fumaric acid and allyl isothiocyanate that target lactic acid bacteria and yeasts, J. Food Sci. 75 (2010) M204–M208, http://dx.doi.org/10.1111/j.1750-3841.2010.01587.x.

[17] M.J. Piercey, G. Mazzanti, S.M. Budge, P.J. Delaquis, A.T. Paulson, L. Truelstrup Hansen, Antimicrobial activity of cyclodextrin entrapped allyl isothiocyanate in a model system and packaged fresh-cut onions, Food Microbiol. 30 (2012) 213–218, http://dx.doi.org/10.1016/j.fm.2011.10.015.

[18] H.-S. Seo, J. Bang, H. Kim, L.R. Beuchat, S.Y. Cho, J.-H. Ryu, Development of an antimicrobial sachet containing encapsulated allyl isothiocyanate to inactivate Escherichia coli O157:H7 on spinach leaves, Int. J. Food Microbiol. 159 (2012) 136–143, http://dx.doi.org/10.1016/j.ijfoodmicro.2012.08.009.

[19] E.A. Siahaan, P. Pendleton, H.-C. Woo, B.-S. Chun, Brown seaweed (Saccharina japonica) as an edible natural delivery matrix for allyl isothiocyanate inhibiting food-borne bacteria, Food Chem. 152 (2014) 11–17, http://dx.doi.org/10.1016/j.foodchem.2013.11.116.

[20] A.E. Wilson, M. Bergaentzlé, F. Bindler, E. Marchioni, A. Lintz, S. Ennahar, In vitro efficacies of various isothiocyanates from cruciferous vegetables as antimicrobial agents against foodborne pathogens and spoilage bacteria, Food Control 30 (2013) 318–324, http://dx.doi.org/10.1016/j.foodcont.2012.07.031.

[21] J. Yun, X. Fan, X. Li, Inactivation of Salmonella enterica serovar typhimurium and quality maintenance of cherry tomatoes treated with gaseous essential oils, J. Food Sci. 78 (2013) M458–M464, http://dx.doi.org/10.1111/1750-3841.12052.

[22] N. Aissani, P. Tedeschi, A. Maietti, V. Brandolini, V.L. Garau, P. Caboni, Nematicidal activity of allylisothiocyanate from horseradish (Armoracia rusticana) roots against Meloidogyne incognita, J. Agric. Food Chem. 61 (2013) 4723–4727, http://dx.doi.org/10.1021/jf4008949.

[23] G. Meca, F.B. Luciano, T. Zhou, R. Tsao, J. Manes, Chemical reduction of the mycotoxin beauvericin using allyl isothiocyanate, Food Chem. Toxicol. 50 (2012) 1755–1762, http://dx.doi.org/10.1016/j.fct.2012.02.070.

[24] C.M. Ranger, M.E. Reding, J.B. Oliver, J.J. Moyseenko, N. Youssef, C.R. Krause, Acute toxicity of plant essential oils to scarab larvae (Coleoptera: Scarabaeidae) and their analysis by gas chromatography–mass spectrometry, J. Econ. Entomol. 106 (2013) 159–167, http://dx.doi.org/10.1603/Ec12319.

[25] D. Steverding, S. Michaels, K.D. Read, In vitro and in vivo studies of trypanocidal activity of dietary isothiocyanates, Planta Med. 80 (2014) 183–186, http://dx.doi.org/10.1055/s-0033-1360262.

[26] A. Bhattacharya, Y. Li, Y. Shi, Y. Zhang, Enhanced inhibition of urinary bladder cancer growth and muscle invasion by allyl isothiocyanate and celecoxib in combination, Carcinogenesis 34 (2013) 2593–2599, http://dx.doi.org/10.1093/carcin/bgt280.

[27] C. Fimognari, E. Turrini, L. Ferruzzi, M. Lenzi, P. Hrelia, Natural isothiocyanates: genotoxic potential versus chemoprevention, Mutat. Res. 750 (2012) 107–131, http://dx.doi.org/10.1016/j.mrrev.2011.12.001.

[28] S.V. Singh, K. Singh, Cancer chemoprevention with dietary isothiocyanates mature for clinical translational research, Carcinogenesis 33 (2012) 1833–1842, http://dx.doi.org/10.1093/carcin/bgs216.

[29] N. Wang, L.Q. Shen, S.X. Qiu, X.Y. Wang, K.W. Wang, J. Hao, M.F. Xu, Analysis of the isothiocyanates present in three Chinese Brassica vegetable seeds and their potential anticancer bioactivities, Eur. Food Res. Technol. 231 (2010) 951–958, http://dx.doi.org/10.1007/s00217-010-1348-x.

[30] F.B. Luciano, F.S. Hosseinian, T. Beta, R.A. Holley, Effect of free-SH containing compounds on allyl isothiocyanate antimicrobial activity against Escherichia coli O157:H7, J. Food Sci. 73 (2008) M214–M220, http://dx.doi.org/10.1111/j.1750-3841.2008.00762.x.

[31] K. Xu, P.J. Thornalley, Studies on the mechanism of the inhibition of human leukaemia cell growth by dietary isothiocyanates and their cysteine adducts in vitro, Biochem. Pharmacol. 60 (2000) 221–231, http://dx.doi.org/10.1016/S0006-2952(00)00319-1.

[32] A. Perin-Martins, J.M. Teixeira, C.H. Tambeli, C.A. Parada, L. Fischer, Mechanisms underlying transient receptor potential ankyrin 1 (TRPA1)-mediated hyperalgesia and edema, J. Peripher. Nerv. Syst. 18 (2013) 62–74, http://dx.doi.org/10.1111/jns5.12010.

[33] C.S. Charron, B.A. Clevidence, G.A. Albaugh, M.H. Kramer, B.T. Vinyard, J.A. Milner, J.A. Novotny, Assessment of DNA damage and repair in adults consuming allyl isothiocyanate or Brassica vegetables, J. Nutr. Biochem. 24 (2013) 894–902, http://dx.doi.org/10.1016/j.jnutbio.2012.06.004.

[34] J.C. Santos, L.R.A. Faroni, A.H. Sousa, R.N.C. Guedes, Fumigant toxicity of allyl isothiocyanate to populations of the red flour beetle Tribolium castaneum, J. Stored Prod. Res. 47 (2011) 238–243, http://dx.doi.org/10.1016/j.jspr.2011.03.004.

[35] Y.-K. Yun, H.-K. Kim, J.-R. Kim, K. Hwang, Y.-J. Ahn, Contact and fumigant toxicity of Armoracia rusticana essential oil, allyl isothiocyanate and related compounds to Dermatophagoides farinae, Pest Manag. Sci. 68 (2012) 788–794, http://dx.doi.org/10.1002/ps.2327.

[36] O. Scott, E. Galicia-Connolly, D. Adams, S. Surette, S. Vohra, J.Y. Yager, The safety of cruciferous plants in humans: a systematic review, J. Biomed. Biotechnol. 2012 (2012) 503241, http://dx.doi.org/10.1155/2012/503241.

[37] EFSA Panel on Food Additives and Nutrient Sources Added to Food (ANS), Scientific opinion on the safety of allyl isothiocyanate for the proposed uses as a food additive, EFSA J. 8 (2010) 1943, http://dx.doi.org/10.2903/j.efsa.2010.1943.

[38] M. Murata, N. Yamashita, S. Inoue, S. Kawanishi, Mechanism of oxidative DNA damage induced by carcinogenic allyl isothiocyanate, Free Radic. Biol. Med. 28 (2000) 797–805, http://dx.doi.org/10.1016/S0891-5849(00)00168-4.

[39] IARC, Some chemicals that cause tumours of the kidney or urinary bladder in rodents and some other substances, IARC Monogr. Eval. Carcinog. Risks Hum. 73 (1999) 37–48.

[40] W.J. Waddell, Comparison of human exposures to selected chemicals with thresholds from NTP carcinogenicity studies in rodents, Hum. Exp. Toxicol. 22 (2003) 501–506, http://dx.doi.org/10.1191/0960327103ht374oa.

[41] D.L. Geiger, L.T. Brooke, D.J. Call, Acute Toxicities of Organic Chemicals to Fathead Minnows (*Pimephales promelas*), Center for Lake Superior Environmental Studies, University of Wisconsin, Superior, WI, USA, 1990.

[42] D.A. Dawson, J.A. Bantle, Development of a reconstituted water medium and preliminary validation of the Frog Embryo Teratogenesis Assay-*Xenopus* (FETAX), J. Appl. Toxicol. 7 (1987) 237–244, http://dx.doi.org/10.1002/jat.2550070403.

[43] ASTM International, ASTM E1439-12: Standard Guide for Conducting the Frog Embryo Teratogenesis Assay-*Xenopus* (FETAX), ASTM International, West Conshohocken, PA, 2013.

[44] J.A. Bantle, R.A. Finch, D.T. Burton, D.J. Fort, D.A. Dawson, G. Linder, J.R. Rayburn, M. Hull, M. Kumsher-King, A.M. Gaudet-Hull, S.D. Turley, FETAX interlaboratory validation study: phase III – Part 1 testing, J. Appl. Toxicol. 16 (1996) 517–528, http://dx.doi.org/10.1002/(SICI)1099-1263(199611)16:6<517::AID-JAT385>3.0.CO;2-R.

[45] J.A. Bantle, R.A. Finch, D.J. Fort, E.L. Stover, M. Hull, M. Kumsher-King, A.M. Gaudet-Hull, Phase III interlaboratory study of FETAX – Part 3. FETAX validation using 12 compounds with and without an exogenous metabolic activation system, J. Appl. Toxicol. 19 (1999) 447–472, http://dx.doi.org/10.1002/(SICI)1099-1263(199911/12)19:6<447::AID-JAT601>3.0.CO;2-4.

[46] J.N. Dumont, J.A. Bantle, G. Linder, The history and development of FETAX (ASTM standard guide, E-1439 on conducting the frog embryo teratogenesis Assay-*Xenopus*), ASTM Spec. Tech. Publ. 1443 (2003) 3–22.

[47] M. Friedman, J.R. Rayburn, J.A. Bantle, Developmental toxicology of potato alkaloids in the frog embryo teratogenesis assay-*Xenopus* (FETAX), Food Chem. Toxicol. 29 (1991) 537–547, http://dx.doi.org/10.1016/0278-6915(91)90046-A.

[48] M. Friedman, J.R. Rayburn, J.A. Bantle, Structural relationships and developmental toxicity of *Solanum* alkaloids in the frog embryo teratogenesis assay-*Xenopus*, J. Agric. Food Chem. 40 (1992) 1617–1624, http://dx.doi.org/10.1021/Jf00021a029.

[49] J.R. Rayburn, J.A. Bantle, M. Friedman, Role of carbohydrate side chains of potato glycoalkaloids in developmental toxicity, J. Agric. Food Chem. 42 (1994) 1511–1515, http://dx.doi.org/10.1021/Jf00043a022.

[50] J.R. Rayburn, M. Friedman, J.A. Bantle, Synergistic interaction of glycoalkaloids α-chaconine and α-solanine on developmental toxicity in *Xenopus* embryos, Food Chem. Toxicol. 33 (1995) 1013–1019, http://dx.doi.org/10.1016/0278-6915(95)00081-X.

[51] J.R. Rayburn, M. Friedman, L-Cysteine, N-acetyl-L-cysteine, and glutathione protect *Xenopus laevis* embryos against acrylamide-induced malformations and mortality in the Frog Embryo Teratogenesis Assay, J. Agric. Food Chem. 58 (2010) 11172–11178, http://dx.doi.org/10.1021/jf1023998.

[52] J.A. Bantle, ASTM committee E-47 on biological effects and environmental fate. Subcommittee E47.01 on aquatic toxicology, in: Atlas of Abnormalities: A Guide for the Performance of FETAX, Printing Services, Oklahoma State University, Stillwater, OK, 1991.