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Residue analyses and exposure assessment of the Irish population to nitrofuran metabolites from different food commodities in 2009-2010

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An exposure assessment to nitrofuran residues was performed for three human populations (adults, teenagers and children), based on residue analyses of foods of animal origin (liver, honey, eggs and aquaculture) covering the two year period 2009-2010. The occurrence of nitrofuran metabolites in food on the Irish market has been determined for the selected period using the data from Ireland's National Food Residue Database (NFRD) and results obtained from the analysis of retail samples (aquaculture and honey). Laboratory analyses of residues were performed by methods validated in accordance with Commission Decision 2002/657/EC regarding performance of analytical method and results interpretation. Semicarbazide (SEM) was the contaminant most frequently identified and its content ranged from 0.09 to 1.27 μg kg^{-1}. SEM is currently used as a marker of nitrofuran abuse, but may also occur from other sources. The presence of nitrofuran metabolite 3-amino-2-oxazolidinone (AOZ) was detected in two aquaculture samples (prawns) at 1.63 and 1.14 μg kg^{-1}, but such a low number of positive cases did not present sufficient data for a full AOZ exposure assessment. Therefore, the evaluation of exposure has been focused on SEM containing food groups only. Exposure assessments were completed using a probabilistic approach that generated ten iterations. The results of both the upper and lower bound exposure assessments demonstrate that SEM exposure for Irish adults, teenagers and children from selected food commodities are well below EFSA-estimated safe levels.

Keywords: Probabilistic exposure assessment; nitrofuran metabolites; residue determination; UHPLC-MS/MS; liver; aquaculture; honey; food consumption database; semicarbazide.

Introduction

Nitrofurans are synthetic antibacterials that have been used worldwide to treat infections caused by bacteria and protozoa in swine, cattle, poultry, rabbits and fish (Kahn 2010). The use of nitrofurans in food producing animals was banned in the EU since the mid-90s because of their potential to cause harmful effects to human health (European Commission 1990). Nitrofurans are listed in annex IV of Commission Regulation (EU) No. 37/20 10, as pharmacologically active substances for which no maximum residue level (MRL) in food can be established.

Nitrofurans are rapidly metabolised after administration and parent compounds usually cannot be detected in animal tissue 24 hours after application (Nouws and Laurensen 1990). However, nitrofurans form protein-bound metabolites that can persist in animal tissue for many months after treatment and can be released under acidic conditions in the consumer's stomach (Hoogenboom et al. 1991; Horne et al. 1996; McCracken et al. 1997). In order to monitor illegal use of nitrofurans, 3-Amino-2-oxazolidone (AOZ), 3 -amino-5-morpholino-methyl- 1,3 -oxazolidin-2-one (AMOZ), 1 -aminohydantoin (AHD) and semicarbazide (SEM) have been established as marker residues for furazolidone, furaltadone, nitrofurantoin and nitrofurazone, respectively. In the early 2000s, an analytical test was developed to detect protein-bound nitrofuran antibiotic residues in food as part of the EU project FoodBRAND (Cooper et al. 2005). This method was based on the detection of nitrophenyl derivatives (NPAHD, NPAMOZ, NPAOZ and NPSEM) of nitrofurans by liquid chromatography coupled to tandem mass spectrometry, after release of protein bound residues by acid hydrolysis and derivatisation with 2-nitrobenzaldehyde (Cooper et al. 2005; Vass et al. 2008). Most of the present confirmatory methods are still based on this principle of detection.

The MRPL level of 1 $\mu g\ kg^{-1}$ currently in use for nitrofuran metabolites has been established by Commission Decision 2003/181/EC in March 2003. This level is

53 applied as a reference point of action for imports from third countries, as laid down in
54 Commission Decision 2005/34/EC.
55 In order to collect and quickly distribute monitoring data, the European Commission
56 established a network, namely the Rapid Alert System for Food and Feed (RASFF).
57 Although the RASFF notifications indicate that there has been a significant decrease
58 in nitrofuran positives since it was established in 2002, there still remains ongoing
59 evidence of illegal use of these substances. A database search for non-compliant
60 results in EU covering period 2009-2010 produced a list of 106 notifications. The
61 highest frequency of positives (97 notifications) was found in seafood (crustaceans
62 etc.) and less frequently in fish, honey, meat and poultry (9 notifications in total).
63 About 86% of all nitrofuran notifications in this period came from the detection of
64 semicarbazide (SEM), mostly in shrimps. Caution should be applied when
65 interpreting SEM positive results because the validity of SEM as an unambiguous
66 marker for nitrofurazone abuse has been previously questioned with regards to other
67 contamination sources (Hruska and Franek 2009). It has been demonstrated that the
68 presence of SEM in processed foods was caused, in the past, by thermal
69 decomposition of a blowing agent, azodicarbonamide, in jar gaskets before its use was
70 banned (de Souza et al. 2005). In other cases, it has been reported that SEM can occur
71 naturally in the food binding agent carrageenan and levels increased by several orders
72 of magnitude following hypochlorite treatment (Hoenicke et al. 2004).
73
74 The objective of this research was to estimate the exposure of the Irish population to
75 nitrofuran metabolite residues from different food commodities during the 2009 to
76 2010 period. This work is based on data included in Ireland's National Food Residue
77 Database (NFRD) and supplementary retail survey data to examine the potential

exposure of three Irish human populations: adults (18-90 years), teenagers (13-17 years) and children (5-12 years) to residues of nitrofuran metabolites arising from the consumption of products of aquaculture, liver, honey and eggs. The NFRD database is publicly available online and contains results of chemical food safety monitoring in Ireland (NFRD 2005). Nitrofurans and SEM were selected for exposure analysis because they have been detected in food samples in recent years. The exposure analysis carried out in this paper interrogates these data and puts it in context from a food safety perspective.

Materials and methods

Standards, reagents and apparatus for residue analyses

NF metabolites (AOZ, AMOZ and AHD), nitrophenyl (NP) derivatives: 3-((2-Nitro-benzylidene)-amino)-oxazolidin-2-one (NPAOZ), 5-Morpholin-4-ylmethyl-3 -((2-nitro-benzylidene)-amino)-oxazolidin-2-one (NPAMOZ), 1 -((2-Nitrobenzylidene)-amino)-imidazolidine-2,4-dione (NPAHD), 2-Nitro-benzaledehyde-semicarbazone (NPSEM) and isotopically labelled internal standards (AMOZ-D_5, AOZ-D_4, $^{13}C^{15}N_2$-SEM and $^{13}C_3$-AHD) were all obtained from Witega, Berlin, Germany. Semicarbazide (SEM) (Vetranal grade), 2-nitrobenzaldehyde (2-NBA), ammonium acetate (MS grade) and 99.5% deuterated methanol were purchased from Sigma Aldrich. Individual primary stock solutions of NF metabolites and their NP derivatives were prepared at a concentration of 50 mg L^{-1} (free metabolite equivalents) in methanol. Internal standards were prepared at a concentration of 50 mg L^{-1} in deuterated methanol. All standard solutions in this work were stored at -20^{o}C. Primary stock solutions were found to be stable for one year. Working standards were prepared daily from intermediate standard solutions (1 mg L^{-1}) at a concentration of 50 µg L^{-1} (free

103 metabolites, NP derivatives and labelled standards). Ultra-pure water (18.2 MΩ) was
104 generated in the laboratory by using a Milli-Q Plus water purification system.
105 Methanol and ethyl acetate (EtOAc) (both HPLC grade), were obtained from BDH
106 Chemicals Ltd. (Poole, UK). Ethanol was obtained from Merck (Germany), and
107 diethylether and cyclohexane (99.5%) from Lab-Scan (Ireland). 0.1M HCl was
108 prepared by diluting 8.6 mL of conc. HCl to 1000 mL with water. 1M NaOH was
109 prepared by dissolving 40 g of sodium hydroxide pellets (Analar Grade, BDH) in
110 water and making up to 1L. Trisodium phosphate buffer 0.3M was prepared by
111 dissolving 11.4 g of $Na_3PO_4.12H_2O$ to 100 mL with water. pH test strips 4.5 – 10.0
112 were obtained from Sigma Aldrich. A Dispensette® lll solvent dispenser (Brand
113 GMBH + CO KG; Wertheim Germany) was used for aliquoting EtOAc. A Mistral
114 3000i centrifuge (MSE; London, UK), TopMix multi-vortexer (Fisher Scientific;
115 Dublin, Ireland) and 13 mm Whatman ReZist™ PTFE syringe filters (0.22 µm and
116 0.45 µm) were obtained from Fisher Scientific (Dublin, Ireland). Oasis HLB SPE
117 cartridges (60 mg, 3 mL) were obtained from Waters Corporation.
118
119 ***Sample preparation***
120 *Aquaculture and liver samples*
121 Samples were weighed in 50 ml PP centrifuge tubes (1g) and homogenized for 1 min
122 with ice cold methanol (8 mL) and water (1 mL). After centrifugation (2030 x*g*, 4°C,
123 10 min), the supernatant was discarded and the sample was repeatedly washed by
124 vortexing (10 s) with ice cold methanol (3 x 4mL), ethanol (2 x 4 mL) and diethyl
125 ether (2 x 4 mL). After solvent evaporation, the dry pellet was broken with a spatula.
126 Internal standard (40 µL), 9 mL of hydrochloric acid (0.1M) and 0.5 mM 2-
127 nitrobenzaldehide in methanol (100 µL) were sequentially added to pellet. Samples

were incubated in a shaking water bath at 37°C (16 h). After cooling to room temperature, samples were neutralised by adding 0.3 M trisodium phosphate buffer (1 mL) and 1M NaOH (385 µL). The pH was checked with test strips (pH 4.5-10) and corrected if necessary to fall in the range pH 6.5 to 7.5. Extraction was performed with EtOAc (18 mL) by shaking samples on a mechanical shaker (20 min). The samples were centrifuged (2030 ×*g*, 10 min) and extracts collected into glass tubes. The extraction was repeated with EtOAc (9 mL) and extracts were combined. The solvent was evaporated under nitrogen at 40°C. The dry extract was reconstituted in 0.5 mL of injection solution (0.5 mM ammonium acetate and methanol 80:20, *v/v*) and vortexed (1 min). Extracts were filtered through 0.2 µm PTFE 13 mm syringe filters into 200 µL vials.

Egg and honey samples

Determination of the total nitrofuran metabolites in eggs was performed in homogenized samples, after removal of the egg shell by a modified method that has been used for the testing of liver and aquaculture. The pre-washing step detailed above was omitted and samples were directly derivatised in acidic conditions. After neutralisation, samples were centrifuged (2030 x*g*, 10 min) and the precipitate removed. The supernatant was purified by vortexing with 6 mlof n-hexane (2 min). After centrifuging (2030 x*g*, 10 min), the hexane layer was discarded and extraction continued with EtOAc, as detailed above for aquaculture and liver. Honey samples were analysed using in-house validated UHPLC-MS/MS methods, as described elsewhere (O'Mahony et al. 2011).

UHPL C-MS/MS analysis

153 UHPLC-MS/MS analytical conditions are described in detail in previously published
154 work (Radovnikovic et al. 2011). Quantification was performed by using extracted
155 matrix calibration curves for each single run. They were obtained by fortifying
156 negative material at five concentration levels (0.2, 0.5, 1, 2 and 5 μg kg^{-1}). Regression
157 analysis of the responses (analyte area divided by internal standard area) was
158 performed using TargetLynx™ software. The acceptable correlation coefficient was r^2
159 >0.995.
160
161 ***Method validation***
162 Analytical methods were validated in-house according to Commission Decision
163 2002/657/EC for each matrix separately (European Commission 2002). Values of
164 CCct and CCJ3 were calculated according to the calibration curve procedure, by using
165 fortified samples. The values of CCa that have been obtained for determination of
166 nitrofuran metabolites in different matrices (liver, egg, honey and aquaculture) are
167 reported in Table 1.
168
169 ***Sources of residue data***
170 *National Residue Control Plan (NRCP)*
171 The sampling strategy was based on guidelines given in Council Directive 96/23/EC
172 on measures to monitor certain substances in live animals and animal products
173 (European Commission 1996). Nitrofurans are listed in Annex I of this directive
174 (group A6), as unauthorised substances whose presence needs to be monitored in
175 bovine, ovine, caprine, porcine and equine products, as well as in aquaculture, eggs
176 and poultry.

Official samples for the NRCP are required to be taken by inspectors at no fixed time and unexpectedly and on no particular day of the week, ensuring that surveillance contains an element of surprise, aimed at detecting illegal administration. Guidelines for sampling levels and frequency are given in Annex IV of the same directive. Collection of samples was performed by authorised inspectors to include target, suspect and random samples as per Commission Decision 98/179/EC (European Commission 1998). Sample quantity, as defined in the NRCP, was sufficient for screening and confirmatory analyses (minimum 400 g for liver and fish, 12 eggs per sample and 400 g of honey).

Retail survey samples

Samples of domestic and imported seafood were taken from the main supermarket retail outlets in frozen and fresh form. The intention was to cover a wide range of raw, cooked and fish-based products in all price ranges. The type of products sampled included raw prawns, cooked and peeled, “ready to eat”, deveined prawns, battered fish meat, wild and farmed fish etc. From a total of 117 samples, 5 (4%) were seafood and products with Irish origin. 15 (13%) samples were imports from the EU and 97 (83%) samples were from non-EU countries.

A total of 249 honey samples were sourced from the main Irish retail outlets in different parts of Ireland, health shops and bee keepers associations etc. They include different varieties labelled as ‘clear’, manuka, acacia, forest, wildflower, clover, eucalyptus, heather and lavender honey, as well as samples of unprocessed honeycomb. There were 19 samples of Irish honey (8%), and 4 samples of blended Irish and non-EU honey (2%). A total of 24 samples were from the EU (10%), while 202 samples were imports from the rest of the world (8 1%).

202

203 ***Selection of residue data for exposure assessment***

204 Nitrofuran residue concentration and prevalence data in different food commodities
205 were extracted from the NFRD for the two year period 2009 – 2010. This selection
206 included 52 hen egg, 22 honey, 316 bovine liver, 62 ovine liver, 104 porcine liver, 80
207 poultry liver, 6 prawn and 67 fish samples. The NFRD data were supplemented with
208 retail survey data, comprising of 249 honey and 117 aquaculture samples. More
209 samples than listed above were tested for NF residue presence, however only
210 food/residue combinations of interest were selected for the purposes of carrying out
211 the exposure assessment, i.e. some matrices which were tested had to be omitted as
212 there were no corresponding consumption data available (e.g. catfish, tilapia and
213 equine liver). The details of sample numbers for each food group are listed in Table 2.

214

215 ***Food consumption data***

216 Intake estimates were based on consumption data collected in the National Adults'
217 Nutrition Survey (NANS), the National Teens' Food Survey (NTFS) and the National
218 Children's Food Survey (NCFS).

219 These surveys investigated the habitual food and beverage consumption, lifestyle,
220 health indicators and attitudes to food and health in a representative Irish sample of
221 1,500 adults aged 18-90 years, 441 teenagers aged 13-17 and 594 children aged 5-12
222 years. This database is one of the most comprehensive of its type in Europe and was
223 established by the Irish Universities Nutrition Alliance (IUNA). The subjects used in
224 this survey were taken on a randomised basis according to the electronic register.

Analysis of the demographic profile was carried out to ensure that the samples were representative of age, sex, geographical location (urban/rural), marital status, social class and socio-economic group. Dietary intake data were obtained using four-day (adults) and seven-day (teenagers and children) semi-weighed food diaries detailing the time, location, cooking method and quantity of each food/drink consumed. Subject height and weight measurements amongst others were also recorded and entered into the database. Detailed survey methodology is available elsewhere (Irish Universities Nutrition Alliance 2012).

For the purposes of estimating exposure to nitrofuran metabolite residues, the food intake data needed to be re-organised into food groups corresponding with those matrices analysed as part of the NRCP and retail survey (e.g. data on honey had to be removed from a generic "honey, syrup, preserves and sweeteners" food group and added to a new "honey total" food group). Food groups were generated for matrices in which positives were found. The food groups utilised in these exposure assessments are listed in Table 3. The same food groups were created for the three dietary surveys, however the number of foods in each group differs among the three assessments for each population. In addition to this, recipe fractions were also utilised. This results in a more accurate measurement of food intake, e.g. a salmon darn will be treated as 100% salmon, whereas a fish pie may only be treated as 10% salmon, depending on the recipe fraction.

Assessment of exposure to nitrofuran metabolites

In estimating the dietary exposure to nitrofuran metabolite residues, there are two basic approaches that may be used in isolation or combination, namely, deterministic

249 and probabilistic. The deterministic approach is based on single-point estimates that
250 are used for each variable within the model (such as an average value or the 97.5th
251 percentile), whereas in the probabilistic approach, the variables are described in terms
252 of distributions (Claeys et al. 2008). The use of distributions allows for all possible
253 values of a variable to be considered in the calculation. This system takes into account
254 every possible value that each variable could have and weights each possible scenario by
255 the probability of its occurrence. Different techniques are available to calculate the
256 outcome distribution, such as the Monte Carlo simulation (Vose 2006), a class of
257 computational algorithms that rely on repeated random sampling to compute their
258 results. A probabilistic model provides the best estimate for consumer exposure to
259 contaminants in the food supply and was used in this study.

260

261 For the exposure assessment, dietary exposure to nitrofuran metabolite residues (µg
262 kg^{-1} bodyweight day^{-1}) was calculated based on individual consumption and
263 bodyweight data, as provided by the three national food surveys, and a combination of
264 residue monitoring data provided by the NFRD, retail survey and CCα values.
265 Nitrofuran metabolites do not decompose significantly after long term storage and are
266 highly stable during conventional cooking procedures (Cooper and Kennedy 2007).
267 Therefore, any possible loss due to processing or cooking were not taken into account.

268 Since no positive samples have been identified containing AHD or AMOZ and there
269 was an insufficient number of positive samples for AOZ (Table 2.), the exposure
270 evaluation has been focused on food groups containing SEM residues only. Two
271 exposure assessments have been carried out for the purpose of estimating exposure of
272 the Irish population to residues of SEM, an upper (scenario A) and lower bound
273 (scenario B) estimate of exposure. In the case of the upper bound estimate, NFRD and

retail survey data were utilised and for samples in which no residue could be detected, 0.5 × CCα (SEM) for the specific matrix was assumed to be the sample residue concentration. This approach aims to avoid an underestimation of exposure as the assumption is made that even though no residue has been detected, it does not necessarily mean that there are zero levels of the residue present. In the case of the lower bound estimate of exposure, the non-detect samples are assigned a residue concentration value of zero.

Once the database for the food groups and the residue samples table based on the SEM metabolite residue data were created, then the basic exposure equation was completed:

$$Exposure = \frac{\sum \text{amount of food consumed x concentration of chemical present}}{\text{Body weight}}$$

The body weight of each subject was also used to express intakes on a per kilogram bodyweight basis. Analyses were completed using software package Creme Food® v3.6.2 (Central Risk Exposure Modelling; Dublin, Ireland) which is a computer program that uses a high-performance cloud computing system to provide an accurate estimate of consumer exposure to various substances. Creme Food® statistical models combine population food consumption patterns with data on residue concentrations in foods and ingredients and deals with variability and uncertainty in the input data. Further details regarding the scope of this software are available elsewhere (Creme *food safety*® 2012). The exposure assessments were run using ten iterations, i.e. the simulated algorithms were repeated ten times to account for the variance in sample concentration values. Exposure from each food group and cumulative exposures to the SEM residues were calculated.

Results and discussion

Confirmatory analyses ofNF metabolites

A total of 1075 samples of aquaculture, liver, eggs and honey were analysed by a confirmatory UHPLC-MS/MS method for detection of four NF metabolites (AHD, AOZ, SEM and AMOZ) that has been validated according to Commission Decision 2002/657/EC. All samples included in the exposure analysis were analysed using the methods in the experimental section of this paper, which were applied in the author's laboratory. The current minimum required performance limit (MRPL) for detection of nitrofurans in tissue is set at 1 $\mu g\ kg^{-1}$, which is based on protein-bound residues (European Commission, 2003). In samples of aquaculture and liver, residues were detected in protein-bound form. The prewashing strategies used for bound nitrofuran residues in tissue are not suitable for egg and honey samples. Instead, nitrofuran residues are determined in these matrices as total metabolites.

The identity of the analytes in matrix was confirmed by their retention time, monitoring of ion ratios of two product ions for each analyte and signal to noise ratio of the transitions with acceptable tolerances defined in Commission Decision 2002/657/EC. To satisfy the requirement for a sufficient number of identification points per compound in low resolution mass-spectrometry, the triple quadrupole was operated in MRM mode, monitoring one parent (pseudomolecular) ion and two daughter ions, which gave the necessary number of identification points per compound (1 point for precursor ion and 1.5 point for each daughter ion providing the 4 points required).

The potential contribution of laboratory consumables to false positive results for SEM was also investigated. It has been previously highlighted that the contact of solvents with certain blown plastics can be a source of trace azodicarbonamide (ADC) that can

323 give signal for SEM content when exposed to heating (de Souza et al. 2005; Stadler et
324 al. 2004). Whenever possible, glassware was used. Additionally pipette tips, septas
325 and paper tissues in the lab were tested as potential SEM sources before being put in
326 use. A reagent blank was introduced as part of each analytical run to eliminate this
327 concern.
328 In the case of compounds that do not have a maximum residue limit (MRL), a non-
329 compliant sample is defined by the laboratory as one where the residue detected was
330 at a concentration in excess of the decision limit (CCα). However, further follow-up
331 investigations are required on-farm for verification purposes because non-compliant
332 results for some substances may occur due to reasons other than illegal use. The CCα
333 is the critical concentration at and above which it can be concluded with an error of
334 probability α that a sample is non-compliant (α is 1 % for compounds listed in Group
335 A of Annex I 96/23/EC) (European Commission 2002). The CCα values that were
336 used in this assessment are obtained by full in-house validation in different matrix and
337 are listed in Table 1.
338 Method performance has been confirmed on an ongoing basis through analyses of
339 various proficiency samples per year, containing nitrofuran metabolites in different
340 matrixes, as per the accreditation scope in the Teagasc laboratory. Satisfactory
341 outcome of undertaken proficiency testing confirms the integrity of NF residue
342 analyses in this laboratory.
343

344 ***Incidence ofNF metabolite residues in foods of animal origin***

345 The 1075 samples analysed in this study resulted in 4300 NF metabolite residue test
346 results. In total there were 19 samples found to contain detectable residues (Table 2),
347 resulting in a 1.8% prevalence of samples containing a detectable residue. SEM was

348 the residue most frequently identified in the positive test samples. The majority of
349 samples found to contain SEM metabolite residues were honey. Other authors report
350 detecting SEM in 21% of honey samples in a survey of commercial honey of various
351 geographic origins (Khong et al. 2004).
352 Presence of SEM is not unambiguous proof of abuse of nitrofurazone, and it has been
353 detected in the past in various food commodities (Hoenicke et al. 2004; Hruska and
354 Franek 2009). Indeed, on-farm investigations carried out by the Irish Department of
355 Agriculture, Food and the Marine did not identify illegal use of nitrofurans in
356 domestic SEM positive cases, indicating contamination comes from sources other
357 than nitrofuran administration.
358 Two aquaculture samples (prawns) were found to contain AOZ above the MRPL (see
359 Table 2), one of which was a border inspection sample.
360

361 ***Exposure assessment results***

362 An exposure assessment was carried out for Irish adults, teenagers and children to
363 SEM, using an upper and lower bound estimate of exposure as previously outlined.
364 Table 4 contains the number of individuals in each of the populations and the
365 estimated number of SEM exposed individuals.

366 *Upper bound estimate of exposure (Scenario A)*

367 The results of the upper bound exposure assessments are presented graphically in
368 Figure 1 for adults (A), teenagers (B) and children (C). As evident from the graph, the
369 exposure levels, even in the case of the upper bound assessment, are extremely low.
370 The calculated SEM exposure for the 95th percentile at 95 confidence interval is only
371 4.19 x 10^{-5} .ig kg^{-1} bw d^{-1} for the adult population, 3.46 x 10^{-5} .ig kg^{-1} bw d^{-1} for

372 teenagers and 3.57x 10^{-5} .ig kg^{-1} bw d^{-1} for children. The positive and negative error
373 values are illustrated for all percentiles.

374 *Lower bound estimate of exposure (Scenario B)*

375 The results of the lower bound exposure assessments are also presented in Figure 1
376 for adults (A), teenagers (B) and children (C), with corresponding error values. As
377 expected the exposure levels are much lower than those seen in the upper bound
378 assessment, as this approach is less conservative. Exposures calculated for the 95th
379 percentile at 95 confidence interval for adults, teenagers and children were 4.65x 10^{-6},
380 3.30x 10^{-6} and 4.83x 10^{-6} .ig kg^{-1} bw d^{-1} respectively (Figure 1).
381 Estimated SEM exposures were all extremely low, even when upper bound
382 assessments are considered.

383 ***Actual food group contributions***

384 The percentage of actual food group contributions to the SEM exposure was
385 calculated based on the upper and lower bound exposure estimates for each of the
386 three populations and the results are illustrated in Figure 2 for adults, teenagers and
387 children respectively.
388 The food groups that contributed the most to the exposure of adults in the upper
389 bound scenario (Figure 2A) were salmon and honey (59.4 and 22.1% respectively).
390 The same scenario indicates the exposure of teenagers to be mostly through honey
391 consumption (46.1%), with salmon as a second contributor (30.4%). The same food
392 groups remain the main exposure contributors in children, under this conservative
393 estimate approach: honey (35.9%) and salmon (3 8.1%).
394 Using the lower bound estimate approach as the basis for calculating the food group
395 contributions to overall exposure, yields results that directly reflect the data presented

396 in the paper (Figure 2B). Using this approach, honey is the highest contributor to adult
397 (49.1%), teenagers (67.6%) and children (55.3%) population exposure.
398 All the figures represented are based on extremely low exposure values. The salmon
399 food group contribution to exposure appears to decrease dramatically from scenario A
400 levels in scenario B. This decrease is due to the fact that the exposure in the lower
401 bound exposure assessment of scenario B is based on a single salmon sample
402 containing a residue of 0.088 µg/kg SEM, see Table 2. In the case of scenario A, the
403 remaining 70 samples would have been substituted with the 0.5*LOD value, thus
404 contributing to the exposure. The honey, prawn and ovine liver food groups contained
405 a greater number of samples containing residues and therefore their contribution to
406 exposure did not decline as significantly from scenario A to scenario B.
407
408 ***Exposure to SEMfrom other sources***
409 Public exposure to SEM is not limited to animal food sources that have been selected
410 for this study. An EFSA report from 2005 highlights that migration of SEM from
411 plastic gaskets represented by far the largest source of exposure (EFSA 2005). The
412 same report details that the average content of SEM in miscellaneous food in jars (121
413 different food products such as fruit, vegetables, jams, pickles, sauces and fish) was 1
414 µg kg^{-1}. SEM residues were a consequence of thermal decomposition of a blowing
415 agent azodicarbonamide (ADC), which was used to make plastic gaskets used in the
416 lids of jars (Ginn et al. 2006). However, production of gaskets using this blowing
417 agent has been phased out since 2006 and exposure to SEM through this route should
418 be reduced significantly, if not eliminated (European Commission 2004). SEM has
419 also been found in bread produced in Third countries, where flour contained ADC as
420 flour improver, starches and egg white powder bleached with hypochlorite (Hoenicke

et al. 2004; Hruska and Franek 2009). Some seaweed and crustaceans have been found to have a naturally high content of SEM (Hoenicke et al. 2004).

EFSA proposed that if food contained an average SEM level of 1.0 μg kg^{-1} (MRPL for SEM), 1 kg of contaminated food would result in a daily exposure of 0.02 μg kg^{-1} bw d^{-1} for a 60 kg bodyweight adult (EFSA 2005). Intake of SEM through alternative sources such as carrageenan (food thickener), resulted in a "worst case" scenario exposure estimate of up to 0.005 μg kg^{-1} bw d^{-1}

The EFSA report concluded that carcinogenicity of SEM was not of concern for human health at the concentrations of SEM encountered in food (EFSA 2005). The report states that, "a large margin of at least 5 orders of magnitude exists between the dose causing tumours in experimental animals and human exposure, including that of infants". Another source reports the no observable adverse effects level (NOAEL) for developmental toxicity in rats at 27 mg SEM kg^{-1} bw day^{-1} (Nestmann et al. 2005). The same study estimated the "worst case" scenario of theoretical infants exposure from ready to eat foods (containing 20 μg kg^{-1} SEM) to be 1.27 μg SEM kg^{-1} bw day^{-1}, and reported it still provided sufficient margin of safety. The results of this study show that all exposure values for SEM from selected food in all three populations are far below the levels that were considered acceptable by EFSA.

Conclusion

Nitrofurans are banned substances and therefore there is need for continuous monitoring of food to prevent their illegal or accidental use in production. Monitoring of these metabolites in foods of animal origin is performed by following sampling programmes defined by the Irish Department of Agriculture, Food and the Marine and EU legislation. In order to provide information on exposure to nitrofuran metabolites

446 from the consumption of foods of animal origin in Ireland in 2009-20 10, residue
447 analyses data from the NFRD and retail samples survey were combined. Currently,
448 the residue monitoring plan in Ireland is based on the analyses of liver and animal
449 plasma as target tissues for detection of illegal use of nitrofurans. Therefore, food
450 groups containing muscle could not be included in the exposure assessment. A total of
451 19 residue containing samples were identified, resulting in 1.8% of positive samples
452 in total number of 1075 samples selected. SEM was the most frequent contaminant
453 identified in positive samples. It has been previously noted that occurrence of SEM in
454 food cannot be considered unambiguous proof of illegal use of nitrofurazone
455 (Hoenicke et al. 2004; Hruska and Franek 2009). As for the other positives identified
456 in this study, two cases of AOZ, identified in two year period, in imported farmed
457 aquaculture (prawns) did not represent sufficient data for appropriate exposure
458 assessment.
459 Probabilistic exposure assessments were carried out using Creme Food software and
460 the results for each subject population (adults, teenagers and children) are summarized
461 in terms of the average daily residue exposure per kilogram of a consumer's
462 bodyweight. The results of both the upper and lower bound exposure assessments
463 clearly indicate that SEM exposure for Irish adults, teenagers and children from
464 consumption of liver, honey, eggs and aquaculture is well below the safe levels
465 indicated by the EFSA from exposure to SEM from variety of sources (EFSA 2005).
466

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474

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Table 1. Values of CCct for various matrices

Analyte	Liver	Honey	Fish	Egg
		[1]CCa (µg kg^{-1})		
Furaltadone as AMOZ	0.073	0.096	0.061	0.079
Furazolidone as AOZ	0.067	0.093	0.041	0.066
Nitrofurantoin as AHD	0.074	0.138	0.057	0.079
Nitrofurazone as SEM	0.064	0.090	0.064	0.074

[1]CCα = Decision limit.

Table 2. Selected NFRD and retail samples from relevant food groups

Sample food group	NRCP samples	Retail samples	Total Sample Number	Result Events Number	Positive samples	Compound identified	Residue content (μg/kg)
Honeytotal	22	249	271	1084	9	SEM	0.541, 0.25, 0.095, 0.09 1, 0.350, 0.253, 1.27, 0.221, 0.227
Aquaculture prawn	6	82	88	352	5	SEM	0.159, 0.206, 0.178
						AOZ	1.626, 1.144
Aquaculture seabass		7	7	28			
Aquaculture trout	20	4	24	96			
Aquaculture salmon	47	24	71	284	1	SEM	0.088
Eggs total	52		52	208			
Liver bovine	316		316	1264			
Liver ovine	62		62	248	4	SEM	0.258, 0.182, 0.172, 0.122
Liver porcine	104		104	416			
Liver poultry	80		80	320			
Total	709	366	1075	4300	19		

Table 3. Food groups created and utilised in the exposure assessment

Population	*Food group*	**Food name**
Adults	Prawns	Prawn Chow Mein; Prawn Vegetable Curry; Prawn & Cream & Veg Pasta Mix; Fish Pie (Cod/P rawns/No Potatoes); Prawns w/ Butter & Garlic; Recipe-Prawns in Filo Pastry; King Prawns in Batter; Prawn Stir Fry (Sweetcorn,Mange,Onion,Carr); Prawn Dumplings; Prawns, raw; Prawns, boiled; Prawns, boiled, weighed with shells; Shrimps, boiled; Shrimps, canned in brine, drained; Pilau, prawn; Curry, prawn, takeaway; Szechuan prawns with vegetables, takeaway; Pork and chicken chow mein
	Salmon	Salmon Baked In Butter; Salmon Fried In Olive Oil; Custom food-Smoked Salmon Pate; Salmon Pie; Salmon Fried in Blended Oil; Salmon, grilled, weighed with bones and skin; Salmon, steamed; Salmon, steamed, weighed with bones and skin; Salmon, smoked; Salmon, pink, canned in brine, flesh and bones, drained; Salmon, red, canned in brine, flesh only, drained; Fish cakes, salmon, homemade; Salmon en croute, retail; Salmon, raw; Salmon, grilled; Salmon, pink, canned in brine, flesh only, drained
	Honey	[illegible] Banana Smoothie (Yogurt,Milk,Honey); Banana & OJ Smoothie (w/Yog); Prawn Dumplings; Honey; Nougat
	Ovine liver	Liver, lamb, fried; liver sausage; beef wellington; pate
Teenagers	Prawns	Prawn crackers, takeaway;Chicken, Shrimp & Veg Stirfry; Prawns, boiled; Prawns, boiled, weighed with shells; Prawns, frozen, raw; Curry, prawn, takeaway; Salmon & Prawn En Croute; Prawns in Filo Pastry; King Prawns in Batter; Prawn Stir Fry (Sweetcorn,Mange,Onion,Carr); Prawn Dumplings; Prawn Chop Suey (7 Veg); Cod, Prawn & Beef Stew; Prawn Cocktail Sauce
	Salmon	Salmon w/ Veg in Stock; Salmon, smoked; Salmon, pink, canned in brine, flesh and bones, drained; Salmon, red, canned in brine, flesh and bones, drained; Salmon en croute, retail; Salmon, grilled; Salmon Baked in Butter; Salmon & Prawn En Croute; Salmon Fried in Blended Oil
	Honey	Honey; Banana Smoothie (Yogurt,Milk,Honey); Raspberry & Banana Smoothie (Low Fat Yog/OJ); Banana & OJ Smoothie (w/Yog); Smoothie (Banana,Grape,OJ); Prawn Dumplings; Cereals mini (Choc/Banana/F&N/Honey); Breakfast Cereals 6 different brands; Honey Nut Shredded Wheat; Nutritional bar
	Ovine liver	Liver pate
Chlidren	Prawns	Prawn crackers, takeaway; Prawn Chow Mein; Prawn Pasta Salad; Prawn Vegetable Curry; Prawn & Cream & Veg Pasta Mix; Prawn & Vegetable Rice w/ Potatoes; Fish Pie (Cod/Prawns/No Potatoes); Prawns w/ Butter & Garlic; Prawns, raw; Prawns, boiled; Prawn Cocktail Sauce; Haddock & Prawn Bake - Count on us; Chicken Prawn & Lemongrass Noodles; Shrimps, boiled, weighed with shells
	Salmon	Fish cakes, salmon, homemade; Salmon Baked In Butter; Salmon Fried in Olive Oil; Salmon Pie; Salmo n Fried in Blended Oil; Salmon & Mayo Spread; Salmon, steamed; Salmon, steamed, weighed with bones and skin; Salmon, smoked; Salmon, pink, canned in brine, flesh and bones, drained; Salmon, red, canned in brine, flesh only, drained; Salmon, red, canned in brine, flesh and bones, drained; Salmon en croute, retail; Salmon, grilled; Salmon, pink, canned in brine, flesh only, drained; Smoked Salmon Pate
	Honey	Honey; Homemade Brown Yeast Bread; Banana Flip (w/ Yogurt, Milk, Honey); Banana & Strawberry Smoothie w / Yogurt & OJ; Chicken Casserole (Orange Juice); Breakfast Cereals 9 different types
	Ovine liver	Liver, lamb, fried; Liver Stew w/ Potatoes; Pate, liver

Table 4. Population pool and estimated number of exposed consumers utilised for exposure assessment

Population	*Scenario*	*Total Population*	*Consumers exposed*
Adults	A	1500	495
	B	1500	29
Teenagers	A	441	99
	B	441	8
Children	A	594	167
	B	594	14

Scenario A; non-detect samples substituted with a samples concentration of 0.5*CCα
Scenario B; non-detect samples retain sample concentration of zero
Total Population: Total number of people in the population analysed.
Consumers: estimated number of food consumers exposed to one or more of the foods containing semicarbazide in the covered period

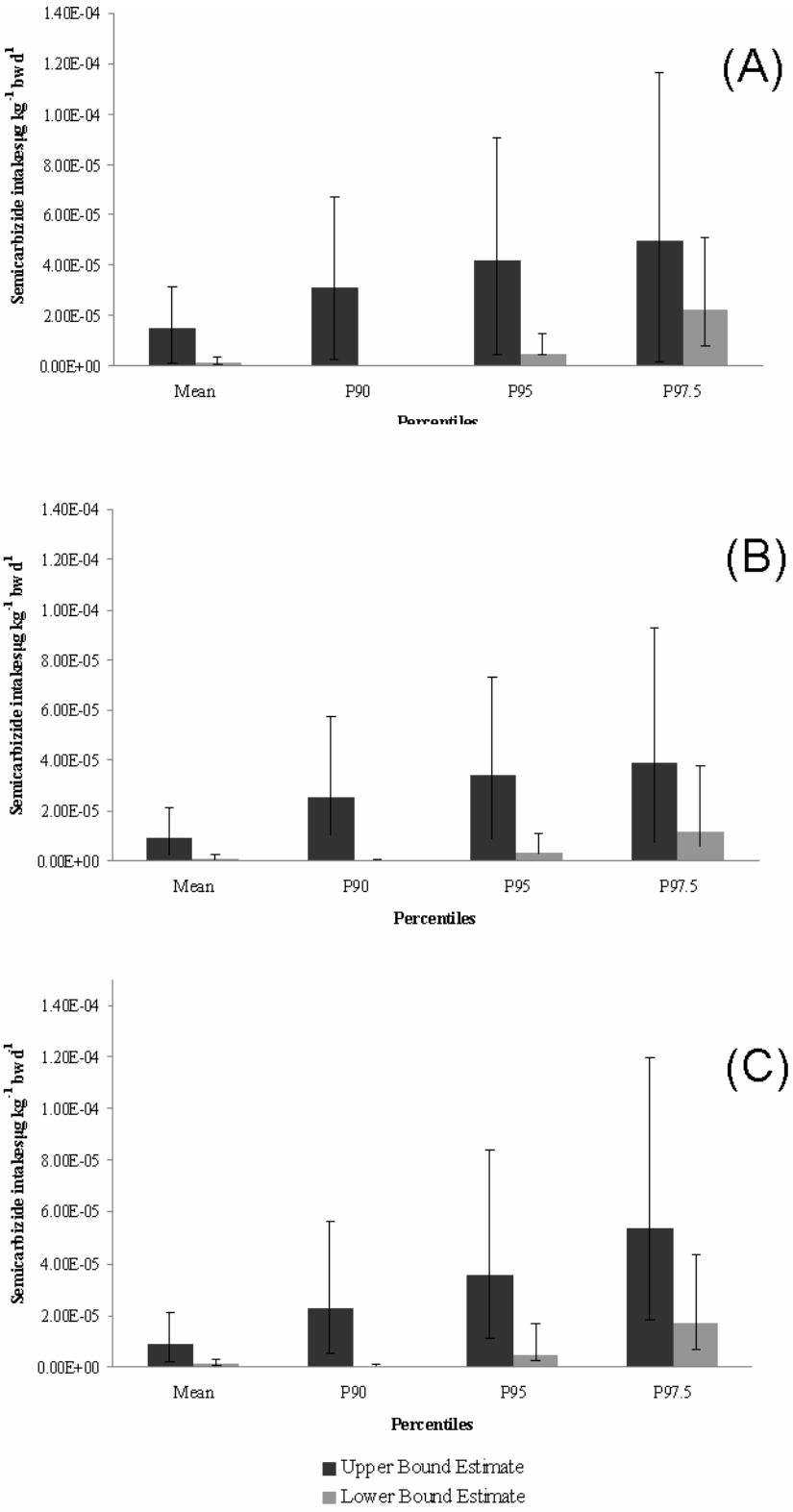
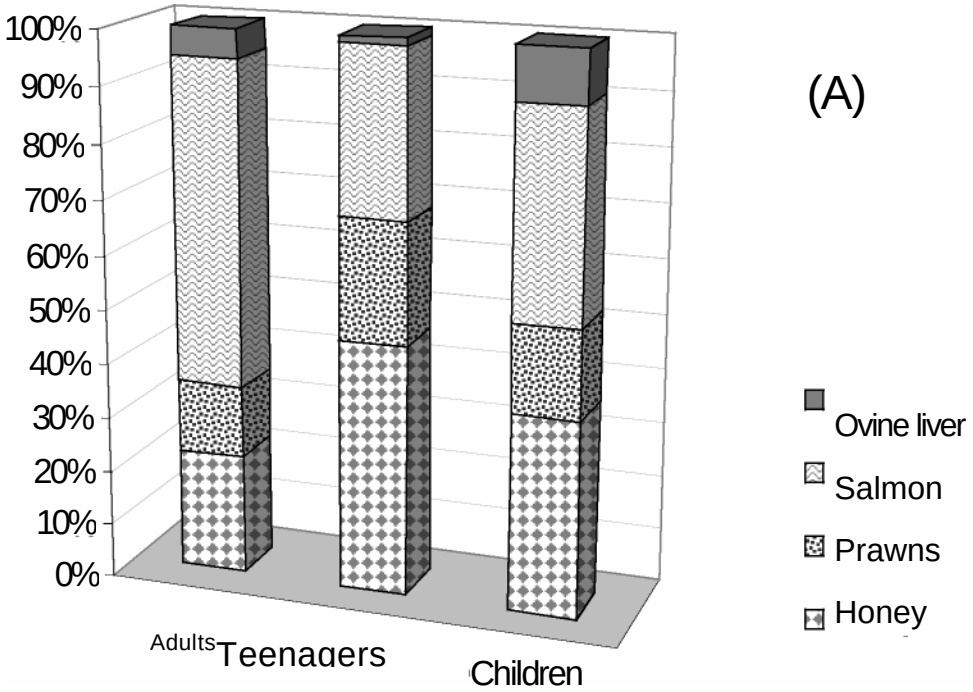


Figure 1. Mean values and SEM for semicarbizide intake(μg/kg/d) for adults (A), teenagers (B) and children (C), using an upper bound estimate and lower bound estimate with 95% confidence intervals.



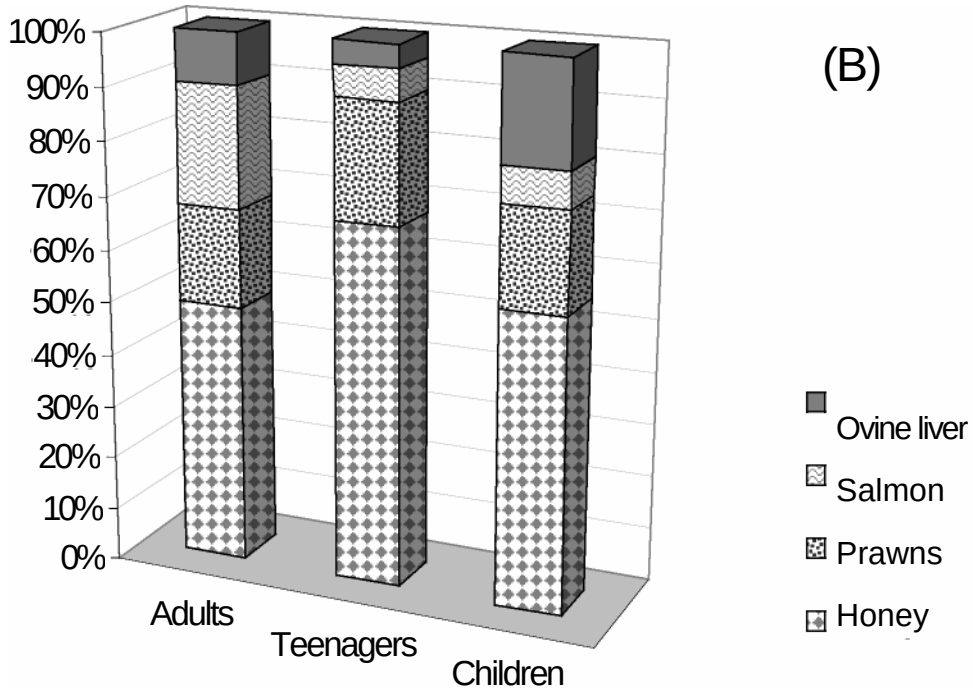


Figure 2. Contribution of food groups to SEM metabolite residue exposure in adults, teenagers and children