



## Survey of 2-Ethylhexanoic acid in baby food.

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**Survey of 2-Ethylhexanoic acid in baby food**

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## Abstract

A solid phase microextraction method was developed and used to perform a survey of 2-ethylhexanoic acid (2-EHA) in 63 samples of baby food from 13 EU countries, Norway and Switzerland. The levels of 2-EHA did not exceed  $3.4 \text{ mg kg}^{-1}$ . The concentrations of 2-EHA in 53 samples (84%) were in the range between less than 0.1 and  $0.5 \text{ mg kg}^{-1}$ . Ten samples showed the presence of the compound in the range between 0.5 and  $3.4 \text{ mg kg}^{-1}$ . An exposure assessment showed that intake of 2-EHA does not exceed the TDI for infants of 6-12 months old except one occurrence when taking the worst-case food consumption data available and the highest level of contamination of the survey. In the other cases the levels of 2-EHA are at 13-fold below the TDI.

*Keywords:* 2-ethylhexanoic acid (2-EHA); solid phase microextraction (SPME); polyacrylate fiber; gas chromatography/mass spectrometry (GC/MS); PVC gasket seals; baby food; tolerable daily intake (TDI); exposure assessment.

**Introduction**

Salts of 2-ethylhexanoic acid are used as thermal plasticisers/stabilisers during the production of PVC sealing of twist-off caps in order to render the seals thermo-stable (HSDB). The PVC gaskets form an airtight seal preventing microbiological and other contamination. Up to now, very limited information is available on the occurrence of 2-EHA in foods. The presence of 2-EHA in baby food and fruit juice packed in glass jars and closed with metal lids, which were sealed by a plastic gaskets was recently detected in a number of the samples from German suppliers and are suspected to be the result of its migration from the gasket seals into the food under the thermal sterilisation (Elss *et al.* 2004). Amounts of 2-EHA in baby food and fruit juices ranged from 0.25 to 3.2 mg kg<sup>-1</sup> and from 0.01 to 0.59 mg l<sup>-1</sup>, respectively (Elss *et al.* 2004). The levels of 2-EHA determined in baby foods on the Norwegian market were from 0.08 to 0.6 mg kg<sup>-1</sup> (NSCFS 2005).

2-EHA is not listed on the positive list for plastics in the contact with food (EC 2006) and there is currently no specific migration limit level for 2-EHA in food contact materials at the EU level. But the substance is included in the Synoptic Document (EC 2005). The latter implies that in the future the compound might be included in the positive list (EC 2006) and the specific or overall migration limit (60 mg kg<sup>-1</sup>) might be applied. Meanwhile it has not been clarified whether the gaskets of metal closures are within the scope of the EU Directive on plastics (EC 2003a). According to the procedure for complying with the provisions of Directive 2002/72/EC on plastic materials and articles in contact with foodstuffs, which is described in Practical Guide

(EC 2003b), metal lids covered with PVC gasket seals are not considered as plastic materials or articles, because they are not made exclusively from plastic. In this case only the general article 3 of the Framework Regulation 1935/2004 (EC 2004) should be applicable as harmonized European legislation.

2-EHA has been included in List “6B” by Scientific Committee of Food (SCF) as the substance suspected of having toxic properties (other than carcinogenic) (EC 2005). 2-EHA is considered negative for mutagenicity but positive for clastogenicity *in vitro* (NSCFS 2005).

The toxicity of 2-EHA on reproduction was studied on rabbits and rats and it was found out that the substance is foetotoxic at high doses (Hendrickx *et al.* 1993). The presence of 2-EHA is linked to peroxisome proliferation of liver cells in rats and mice (Lampen *et al.* 2003, Sundberg *et al.* 1994).

According to Directive 67/548/EEC, Annex VI (EC 1992) the German Federal Institute for Occupational Safety and Health classified 2-EHA as suspected to cause harmful effects on embryos and labelled it as R63, category 3. US Environmental Protection Agency (EPA) announced that 2-ethylhexanoic acid may present an unreasonable risk of oncogenicity, developmental toxicity and subchronic toxicity (EPA 2006).

Several analytical methods for determination of 2-EHA are described in the literature. Elss *et al.* (2004) used Likens-Nickerson simultaneous distillation-extraction (SDE) over 2 h with a pentane-diethyl ether mixture (1:1, v/v) for concentration of 2-EHA

from baby food and fruit juices. Afterwards, the quantification was done by GC/MS (EI) in full scan mode using internal standard - 2-methyl-1-pentanol. For determination of 2-EHA in urine samples Wahl *et al.* (2001) used cleaning procedure with Chromosorb P without prior extraction and subsequent silylation with MTBSTFA at 60 °C for 2 h. Further quantification was performed by GC/MS (EI) in SIM mode using internal standard - *trans*-cinnamic acid. Horn *et al.* (2004) used chloroform for the extraction of 2-EHA from water and sediments. Subsequent analysis was done by GC/MS using RTX-5 capillary column. Few authors reported quantitative headspace SPME analysis of volatiles in foods and beverages (Roberts *et al.* 2000, Ai 1997, Górecki and Pawliszyn 1997, Hawthorne 1992). Also Pinho *et al.* (2002) used a headspace solid phase microextraction (SPME) method in combination with GC/MS for the extraction, identification and quantification of butanoic, hexanoic, octanoic and decanoic acids in ewe cheeses. The method of standard additions and isotope dilution assays (IDA) were used in the latter work for the accurate quantification of the free fatty acids.

In our work we applied external calibration method for headspace SPME analysis using synthetic matrices, which were prepared by choosing carefully the components and quantities in order to match the real samples as much as possible. Also the sampling conditions were optimised in order to reach maximum reproducibility for the standards and the samples. Usually external calibration is not so accurate as standard additions method or IDA due to the fact that a synthetic matrix could hardly match that of authentic sample (Pinho *et al.* 2002). But for our purpose we assumed the compromise between accuracy on one side and moderate amount of the samples on the other side.

## Materials and methods

### *Chemicals*

2-Ethylhexanoic acid ( $\geq 99.5\%$ , Sigma-Aldrich, Milan, Italy) was used as standard material. Ultrapure water prepared using a Milli-Q filter system (Direct Q5 Millipore, Milan, Italy). Metaphosphoric acid (Sigma-Aldrich, Milan, Italy) was used for the preparation of solutions.

### *Samples*

For the calibration 3 types of home-made baby food was prepared by mixing water, rice, vegetables (courgettes, carrots, tomatoes, onions), chicken breast, olive oil and salts at different quantities in order to reproduce commercial types of the food. Real food samples were of several different types: a) meat or fish, rice or pasta and vegetables; b) meat or fish and vegetables; c) rice or pasta and vegetables; d) vegetables. Fat content in each type of the food was from 2.5 to 3.5 %. Sixty three real samples taken for the 2-EHA analysis were part of those which were used for the ESBO chlorohydrins survey (Piccinini *et al.* 2004).

### *Sample preparation*

Each real baby food sample was prepared by weighing 5 g of the food in a 20 ml headspace vial. 5 ml of 5 % metaphosphoric acid solution, 2 ml of distilled water and magnetic stirring bar were added to each vial before sealing hermetically. Real samples were conditioned in the water bath heated at 45 °C for 10 min under magnetic stirring



(IKA, Germany) in order to achieve the partition equilibrium between the liquid and vapour phases. 85  $\mu\text{m}$  polyacrylate SPME fiber (Supelco, USA) was used for the extraction procedure. SPME fiber was exposed to the headspace for 5 min. The analyte was desorbed in the GC injector port at 260  $^{\circ}\text{C}$ . Blank runs were performed between each extraction to ascertain that the fiber is free from 2-EHA. The samples used for calibration were prepared by spiking of home-made baby food at six different concentrations (0.1; 0.2; 0.5; 1; 2 and 4  $\text{mg kg}^{-1}$ ) with 2-EHA by adding 0.05, 0.1, 0.25, 0.5, 1 and 2 ml of 2-EHA solution in water, respectively to 5 g of the food in 20 ml headspace vial. The concentration of 2-EHA was 10.3  $\text{mg l}^{-1}$  (at this concentration 2-EHA is completely soluble in water). Afterwards, 5 ml of 5 % metaphosphoric acid and different amount of water (1.95, 1.9, 1.75, 1.5 and 1 ml) was added in the spiked samples with the concentrations of 2-EHA at 0.1, 0.2, 0.5, 1 and 2  $\text{mg kg}^{-1}$ , respectively. The other conditions for the preparation of the standards were the same as for the real samples. The adjusting of the standard sample volumes with water was performed in order to have the equal (as much as possible) headspace volumes for standard and real samples.

#### *Capillary gas chromatography/mass spectrometry (GC/MS)*

The calibration and real samples were analysed using gas chromatograph CP-3800 (Varian, USA) equipped with 1079 universal capillary injector and a triple quadrupole mass spectrometer 1200L MS/MS (Varian, USA). The narrow bore (0.8 mm ID) deactivated SPME injection liner (Varian, USA) and VF-1ms fused silica capillary column (60 m; 0.25 mm ID; 0.25  $\mu\text{m}$ ) (Varian, USA) were used with the GC/MS. The instrument was operated under the following conditions: injector temperature - 260  $^{\circ}\text{C}$ ,

splitless injection mode for 2 min, then split mode until 3 min with split flow rate - 50 ml/min and finally with split vent opened at the flow rate of 10 ml/min for carrier gas saving. Column oven temperature program: 50 °C (3 min), then 4 °C /min to 220 °C. Transfer line temperature - 250 °C. The massspectromer was operated in EI ionisation (70 eV) mode by scanning the ions from 35 to 150 m/z at the first quadruple. Scan time - 0.5 s; ion source temperature used - 250 °C. Confirmation of the presence of 2-ethylhexanoic acid in the real samples was based on the retention time and on the mass spectrum of 2-EHA. For the quantification the sum of the most intense ions of 2-EHA fragmentation (m/z 73 and m/z 88) was used.

### *Calibration*

The calibration curves for 3 different types of food: a) meat, rice, and vegetables; b) meat and vegetables; c) rice and vegetables were constructed for quantification of the analyte in real samples. 2-EHA was quantified by means of the different calibration curves, which were constructed by analysing spiked home-made food at six different concentrations of 2-EHA (from 0.1 to 4 mg kg<sup>-1</sup>) as described above. Quantification was based on peak area integration. External calibration approach was used for quantitative analysis. Correlation coefficients for the all three curves were higher than 0.99. For each type of the real food sample corresponding calibration curve (in the terms of matrices similarity) was used in order to decrease as much as possible the influence of matrices differences on the quantification results when using external calibration method.

### **Results and Discussion**

The samples analysed were from 13 EU countries: Austria, Belgium, Denmark, France, Germany, Greece, Ireland, Italy, Netherlands, Portugal, Spain, Sweden, United Kingdom, also from Switzerland and Norway. The typical GC/MS total and extracted ion chromatograms of baby food sample contaminated with 2-EHA are shown (see Figure 1).

The results on concentrations of 2-EHA in different type of baby food are summarized (see Table I). It shows that concentration of 2-EHA ranges from non-detectable (less than 0.1 mg kg<sup>-1</sup>) up to 3.4 mg kg<sup>-1</sup>. The concentration of 2-EHA was below 0.1 mg kg<sup>-1</sup> in 27 of the samples (42.9 %). In 26 samples (41.3 %) the amount of 2-EHA ranged between 0.1 and 0.5 mg kg<sup>-1</sup>. The total percentage of the samples with the concentration of 2-EHA from non-detectable (less than 0.1 mg kg<sup>-1</sup>) to 0.5 mg kg<sup>-1</sup> was 84 % (53 out of 63). The later values were consistent with the levels of 2-EHA determined in baby foods on the Norwegian market, which were from 0.08 to 0.6 mg kg<sup>-1</sup> (NSCFS 2005). Three baby food samples from Norway were analysed so far in this survey. The amounts found indicated non-detectable (less than 0.1 mg kg<sup>-1</sup>), 0.3 and 0.4 mg kg<sup>-1</sup> (see Table I), which were within the range of former study (NSCFS 2005). The average concentration of 2-EHA was 0.6 mg kg<sup>-1</sup>, not taking into account the samples in which the amount of 2-EHA was below 0.1 mg kg<sup>-1</sup>. It's in good agreement with the results of twenty baby food samples from 11 German suppliers in which the average concentration of 2-EHA was 0.55 mg kg<sup>-1</sup> (Elss *et al.* 2004). The concentration of 2-EHA in two samples from Germany showed the values: 0.28 and 1.2 mg kg<sup>-1</sup> (see Table I) and were within the range of former study (Elss *et al.* 2004).

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Distribution of concentrations of 2-EHA in the baby food samples is shown (see Figure 2). Comparison of the amounts of 2-EHA found in baby food samples with different content (meat or fish, rice or pasta and vegetables; meat or fish and vegetables; rice or pasta and vegetables; vegetables) shows no distinct correlation. It seems that amount of fat or water and the other constituents in the final product not influences so much for the migration of 2-EHA into the food. Almost exclusively the highest values of 2-EHA were found in the samples from Spain (see Table I). More careful examination showed that 4 samples (out of 7 from Spain, with the concentration of 2-EHA from 1 to 3.4 mg kg<sup>-1</sup>) were delivered from one supplier. This supplier was not found in the information list of food samples, which were delivered from the other countries studied. Also it's worth to note that the concentration of 2-EHA in the two similar samples: "ES-V-05" and "ES-V-07" (see Table I), delivered from the same supplier, differs almost by factor of 10.

Taking into account the findings from this study it is possible to state that the concentration of 2-EHA in the food depends more on technological processes of thermal sterilization and has not so much to do with the content of the food. Also it might be possible that the amount of 2-EHA salts in PVC gasket seals correlates with the concentrations of the substance in the food (from this study it was not evident because the PVC gasket seals were not analysed). The presumption that the concentration of 2-EHA in the food depends on technological process is even more evident from the fact that 43 % of the samples from this study were non-contaminated with 2-EHA.

No tolerable daily intake (TDI) or acceptable daily intake (ADI) for 2-EHA is established by either the EC Scientific Committee for Food (SCF), European Safety Authority (EFSA), Joint FAO/WHO Expert Committee on Food Additives (JECFA) or the U.S. Food and Drug Administration (FDA). The exposure assessment was based on the estimation of TDI - 0.25 mg kg<sup>-1</sup>/day for 2-EHA, which was taken from the opinion published by the Norwegian Committee for Food Safety (NSCFS 2005). The exposure assessment showed that intakes of 2-EHA do not exceed the TDI for infants of 6-12 months old except one occurrence when taking the worst-case food consumption study available and the highest level of contamination of the survey. In the other cases the levels of 2-EHA are at 13-fold below the TDI.

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213-219.

**Figure legends:**

**Figure 1.** GC/MS profiles of typical baby food contaminated with 2-ethylhexanoic acid:  
a) total ion chromatogram (TIC); b) extracted ion chromatogram (IC) (m/z 73+88).

**Figure 2.** Distribution of concentrations of 2-ethylhexanoic acid (2-EHA) in the baby food samples: baby food type (X axis); proportion of the samples, % (Y axis); concentration range of 2-EHA, mg kg<sup>-1</sup> (Z axis).

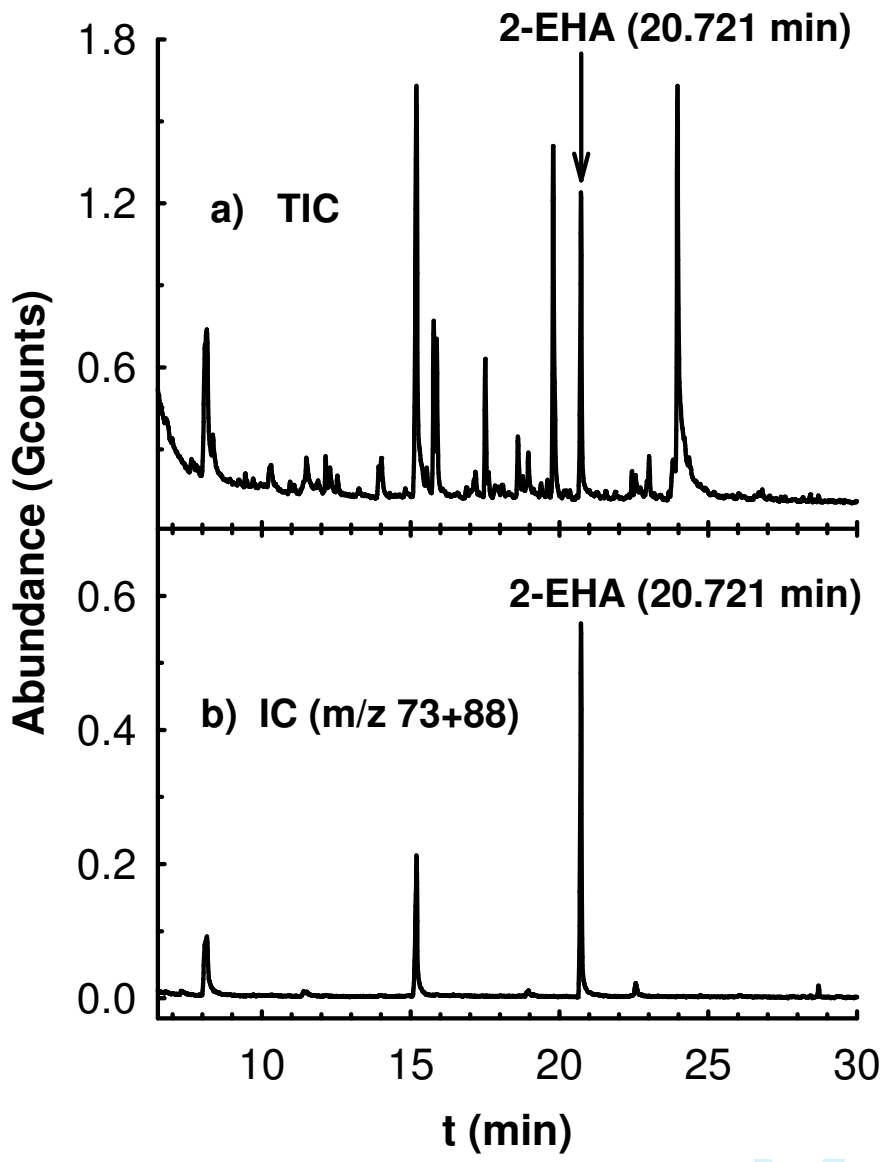


Figure 1.

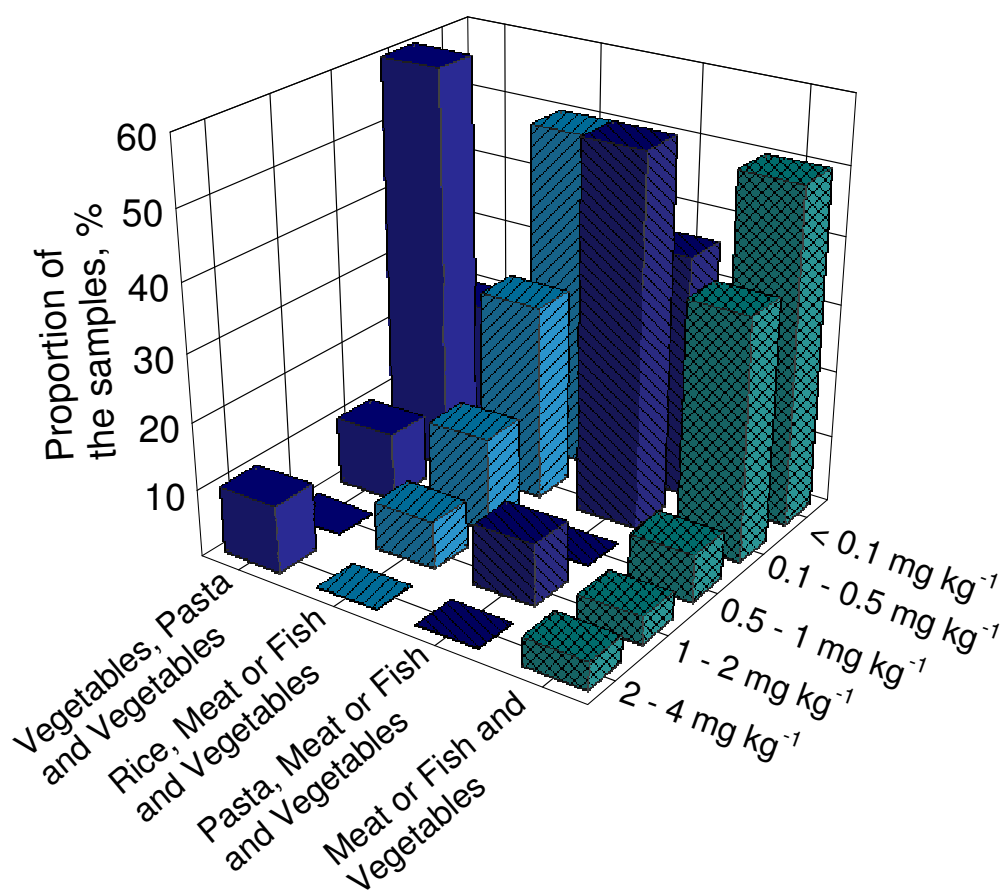


Figure 2.

Table I. The results of 2-ethylhexanoic acid in the baby food samples.

| Baby food sample | Country     | 2-EHA, mg kg <sup>-1</sup> | Baby food sample | Country | 2-EHA, mg kg <sup>-1</sup> | Baby food sample | Country        | 2-EHA, mg kg <sup>-1</sup> |
|------------------|-------------|----------------------------|------------------|---------|----------------------------|------------------|----------------|----------------------------|
| AUT-FPV-07       | Austria     | 0.20                       | EL-MV-07         | Greece  | n.d.                       | IT-MV-05         | Italy          | n.d.                       |
| AUT-MV-01        |             | 0.23                       | ES-FV-13         | Spain   | 2.28                       | NL-FRV-05        | Netherlands    | 0.62                       |
| BE-FV-09         | Belgium     | n.d.                       | ES-MV-11         |         | 1.94                       | NL-FRV-10        |                | n.d.                       |
| BE-MPV-01        |             | 0.29                       | ES-FRV-15        |         | 1.62                       | NL-FV-04         |                | 0.47                       |
| BE-MPV-10        |             | 0.16                       | ES-MRV-12        |         | 0.78                       | NL-MRV-03        |                | 0.23                       |
| BE-MV-05         |             | n.d.                       | ES-V-01          |         | 0.32                       | NL-MV-01         |                | n.d.                       |
| BE-PV-08         |             | n.d.                       | ES-V-05          |         | 3.40                       | NL-MV-14         |                | n.d.                       |
| BE-V-12          |             | 0.30                       | ES-V-07          |         | 0.36                       | N-MV-01          | Norway         | n.d.                       |
| CH-MRV-05        | Switzerland | n.d.                       | F-ChV-23         | France  | 0.71                       | N-MV-13          |                | 0.41                       |
| CH-MV-01         |             | 0.35                       | F-FV-15          |         | 0.46                       | N-V-11           | Portugal       | 0.29                       |
| CH-MV-02         |             | 0.38                       | F-MRV-10         |         | 0.22                       | PT-FV-08         |                | 0.32                       |
| CH-MV-12         |             | 0.31                       | F-MRV-19         |         | 0.21                       | PT-MV-02         | Sweden         | n.d.                       |
| CH-PMV-14        |             | 0.15                       | F-MV-02          |         | 0.66                       | S-MPV-25         |                | 0.22                       |
| CH-PV-10         |             | 0.13                       | F-MV-03          |         | n.d.                       | S-MRV-18         |                | n.d.                       |
| DE-FPV-10        | Germany     | 1.19                       | F-MV-04          |         | 0.43                       | S-MRV-37         | United Kingdom | n.d.                       |
| DE-MV-04         |             | 0.28                       | F-MRV-19         |         | n.d.                       | S-MV-28          |                | n.d.                       |
| DK-MPV-12        | Denmark     | n.d.                       | F-V-08           |         | 0.60                       | UK-FPV-01        |                | n.d.                       |
| DK-MPV-13        |             | 0.28                       | F-V-20           |         | n.d.                       | UK-MRV-12        |                | n.d.                       |
| DK-MRV-07        |             | 0.43                       | IRL-MV-03        | Ireland | n.d.                       | UK-MV-06         |                | n.d.                       |
| DK-MV-01         | Greece      | n.d.                       | IT-MPV-17        | Italy   | n.d.                       | UK-MV-10         |                | n.d.                       |
| EL-MPV-01        |             | n.d.                       | IT-MRV-18        |         | n.d.                       | UK-PV-05         |                | 0.20                       |

n.d. – not detectable (less than 0.1 mg kg<sup>-1</sup>).