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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 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

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4 (EC 2003b), metal lids covered with PVC gasket seals are not considered as plastic  
5 materials or articles, because they are not made exclusively from plastic. In this case  
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7 only the general article 3 of the Framework Regulation 1935/2004 (EC 2004) should be  
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9 applicable as harmonized European legislation.  
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16 2-EHA has been included in List “6B” by Scientific Committee of Food (SCF) as the  
17 substance suspected of having toxic properties (other than carcinogenic) (EC 2005).  
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19 2-EHA is considered negative for mutagenicity but positive for clastogenicity *in vitro*  
20 (NSCFS 2005).  
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28 The toxicity of 2-EHA on reproduction was studied on rabbits and rats and it was found  
29 out that the substance is foetotoxic at high doses (Hendrickx *et al.* 1993). The presence  
30 of 2-EHA is linked to peroxisome proliferation of liver cells in rats and mice (Lampen  
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*et al.* 2003, Sundberg *et al.* 1994).

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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).

54 Several analytical methods for determination of 2-EHA are described in the literature.  
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56 Elss *et al.* (2004) used Likens-Nickerson simultaneous distillation-extraction (SDE)  
57 over 2 h with a pentane-diethyl ether mixture (1:1, v/v) for concentration of 2-EHA  
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4 from baby food and fruit juices. Afterwards, the quantification was done by GC/MS  
5 (EI) in full scan mode using internal standard - 2-methyl-1-pentanol. For determination  
6 of 2-EHA in urine samples Wahl *et al.* (2001) used cleaning procedure with  
7 Chromosorb P without prior extraction and subsequent silylation with MTBSTFA at 60  
8 °C for 2 h. Further quantification was performed by GC/MS (EI) in SIM mode using  
9 internal standard - *trans*-cinnamic acid. Horn *et al.* (2004) used chloroform for the  
10 extraction of 2-EHA from water and sediments. Subsequent analysis was done by  
11 GC/MS using RTX-5 capillary column. Few authors reported quantitative headspace  
12 SPME analysis of volatiles in foods and beverages (Roberts *et al.* 2000, Ai 1997,  
13 Górecki and Pawliszyn 1997, Hawthorne 1992). Also Pinho *et al.* (2002) used a  
14 headspace solid phase microextraction (SPME) method in combination with GC/MS for  
15 the extraction, identification and quantification of butanoic, hexanoic, octanoic and  
16 decanoic acids in ewe cheeses. The method of standard additions and isotope dilution  
17 assays (IDA) were used in the latter work for the accurate quantification of the free fatty  
18 acids.  
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42 In our work we applied external calibration method for headspace SPME analysis using  
43 synthetic matrices, which were prepared by choosing carefully the components and  
44 quantities in order to match the real samples as much as possible. Also the sampling  
45 conditions were optimised in order to reach maximum reproducibility for the standards  
46 and the samples. Usually external calibration is not so accurate as standard additions  
47 method or IDA due to the fact that a synthetic matrix could hardly match that of  
48 authentic sample (Pinho *et al.* 2002). But for our purpose we assumed the compromise  
49 between accuracy on one side and moderate amount of the samples on the other side.  
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## 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



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4 (IKA, Germany) in order to achieve the partition equilibrium between the liquid and  
5 vapour phases. 85  $\mu\text{m}$  polyacrylate SPME fiber (Supelco, USA) was used for the  
6 extraction procedure. SPME fiber was exposed to the headspace for 5 min. The analyte  
7 was desorbed in the GC injector port at 260  $^{\circ}\text{C}$ . Blank runs were performed between  
8 each extraction to ascertain that the fiber is free from 2-EHA. The samples used for  
9 calibration were prepared by spiking of home-made baby food at six different  
10 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,  
11 0.5, 1 and 2 ml of 2-EHA solution in water, respectively to 5 g of the food in 20 ml  
12 headspace vial. The concentration of 2-EHA was 10.3  $\text{mg l}^{-1}$  (at this concentration  
13 2-EHA is completely soluble in water). Afterwards, 5 ml of 5 % metaphosphoric acid  
14 and different amount of water (1.95, 1.9, 1.75, 1.5 and 1 ml) was added in the spiked  
15 samples with the concentrations of 2-EHA at 0.1, 0.2, 0.5, 1 and 2  $\text{mg kg}^{-1}$ , respectively.  
16 The other conditions for the preparation of the standards were the same as for the real  
17 samples. The adjusting of the standard sample volumes with water was performed in  
18 order to have the equal (as much as possible) headspace volumes for standard and real  
19 samples.

#### 40 41 42 43 44 45 *Capillary gas chromatography/mass spectrometry (GC/MS)*

46 The calibration and real samples were analysed using gas chromatograph CP-3800  
47 (Varian, USA) equipped with 1079 universal capillary injector and a triple quadruple  
48 mass spectrometer 1200L MS/MS (Varian, USA). The narrow bore (0.8 mm ID)  
49 deactivated SPME injection liner (Varian, USA) and VF-1ms fused silica capillary  
50 column (60 m; 0.25 mm ID; 0.25  $\mu\text{m}$ ) (Varian, USA) were used with the GC/MS. The  
51 instrument was operated under the following conditions: injector temperature - 260  $^{\circ}\text{C}$ ,  
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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 mass spectrometer was operated in EI ionisation (70 eV) mode by scanning the ions from 35 to 150 m/z at the first quadrupole. 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**

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4 The samples analysed were from 13 EU countries: Austria, Belgium, Denmark, France,  
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6 Germany, Greece, Ireland, Italy, Netherlands, Portugal, Spain, Sweden, United  
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8 Kingdom, also from Switzerland and Norway. The typical GC/MS total and extracted  
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10 ion chromatograms of baby food sample contaminated with 2-EHA are shown (see  
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12 Figure 1).  
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18 The results on concentrations of 2-EHA in different type of baby food are summarized  
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20 (see Table I). It shows that concentration of 2-EHA ranges from non-detectable (less  
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22 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>  
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24 in 27 of the samples (42.9 %). In 26 samples (41.3 %) the amount of 2-EHA ranged  
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26 between 0.1 and 0.5 mg kg<sup>-1</sup>. The total percentage of the samples with the concentration  
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28 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  
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30 of 63). The later values were consistent with the levels of 2-EHA determined in baby  
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32 foods on the Norwegian market, which were from 0.08 to 0.6 mg kg<sup>-1</sup> (NSCFS 2005).  
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34 Three baby food samples from Norway were analysed so far in this survey. The  
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36 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  
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38 Table I), which were within the range of former study (NSCFS 2005). The average  
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40 concentration of 2-EHA was 0.6 mg kg<sup>-1</sup>, not taking into account the samples in which  
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42 the amount of 2-EHA was below 0.1 mg kg<sup>-1</sup>. It's in good agreement with the results of  
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44 twenty baby food samples from 11 German suppliers in which the average  
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46 concentration of 2-EHA was 0.55 mg kg<sup>-1</sup> (Elss *et al.* 2004). The concentration of  
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48 2-EHA in two samples from Germany showed the values: 0.28 and 1.2 mg kg<sup>-1</sup> (see  
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50 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.

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

31 We thanks all the countries, who provided baby food samples for this survey.  
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**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).

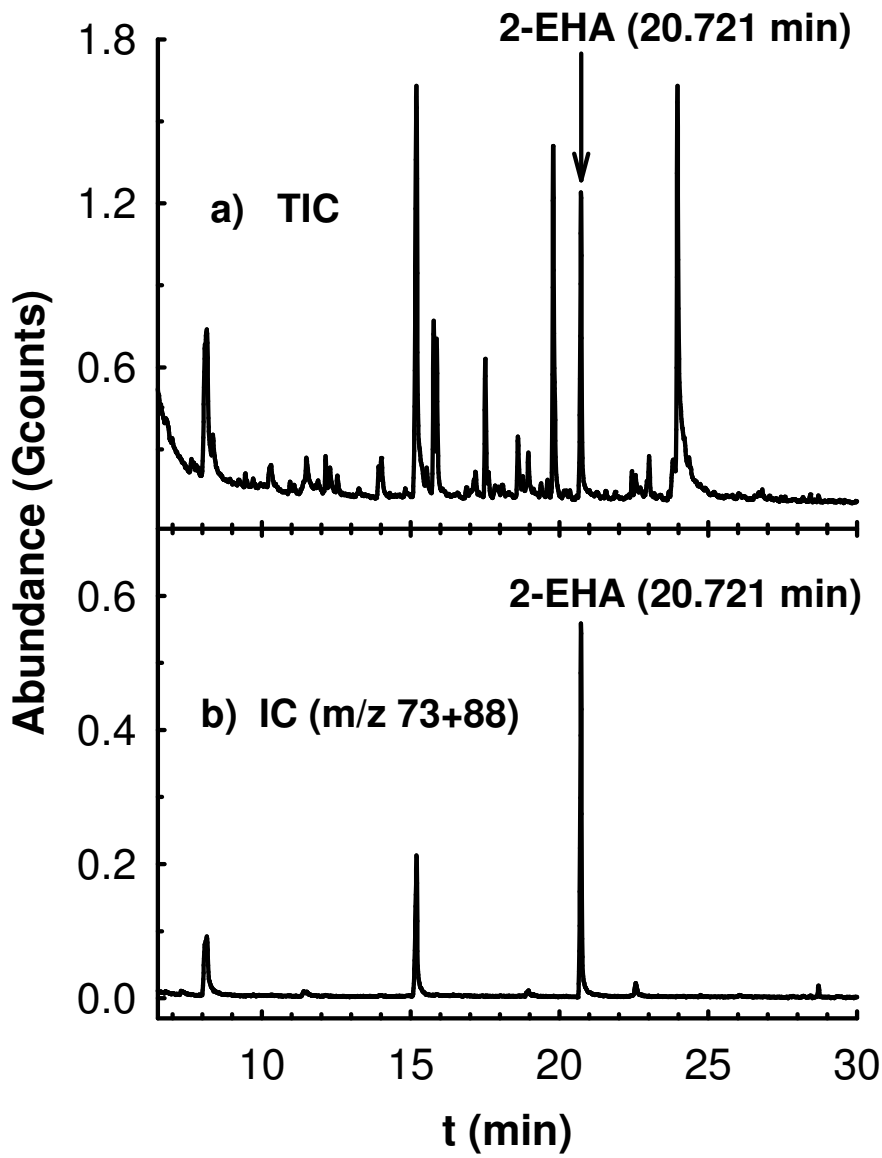


Figure 1.

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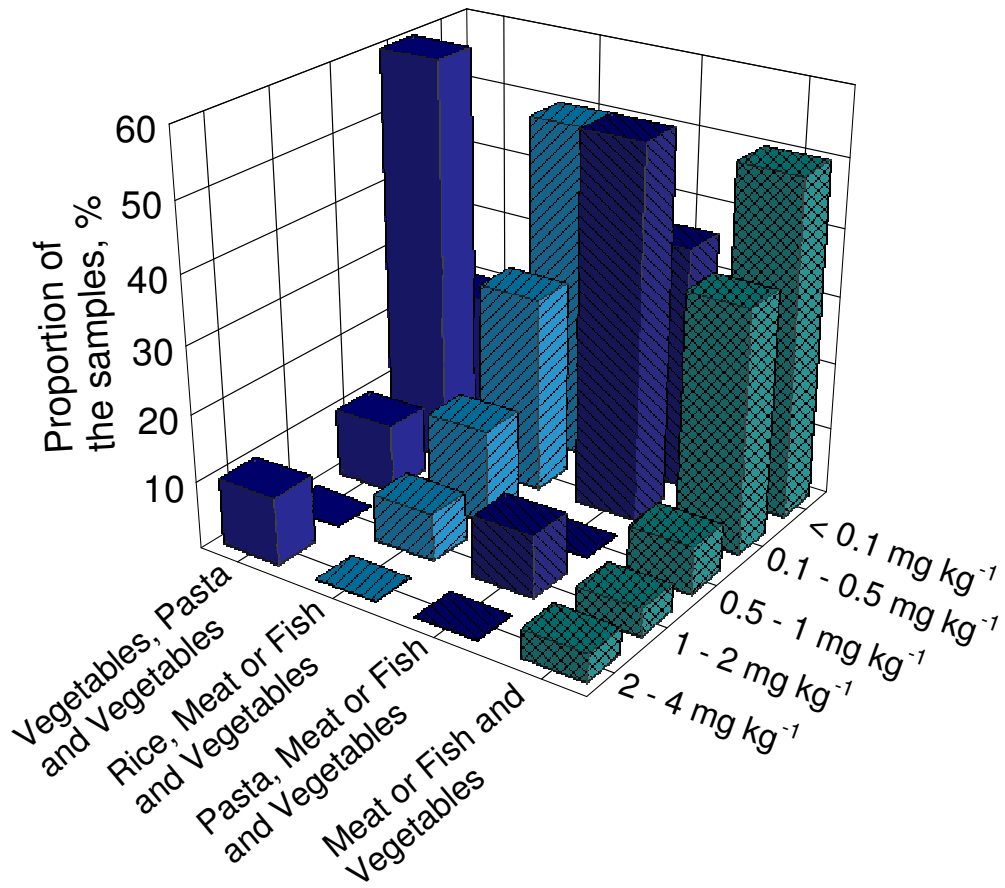


Figure 2.

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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	0.71	N-MV-13	0.41		
CH-MV-01		0.35	F-FV-15	0.46	N-V-11	0.29		
CH-MV-02		0.38	F-MRV-10	0.22	PT-FV-08	Portugal	0.32	
CH-MV-12		0.31	F-MRV-19	0.21	PT-MV-02		n.d.	
CH-PMV-14		0.15	F-MV-02	0.66	S-MPV-25	Sweden	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		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	United Kingdom	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	n.d.	IT-MPV-17	Italy	n.d.	UK-MV-10		n.d.	
EL-MPV-01	Greece	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>).