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**Estimated intake of intense sweeteners from non-alcoholic beverages in  
Denmark**

For Peer Review Only

**Abstract**

In 1999 116 samples of non-alcoholic beverages were analysed for the intense sweeteners cyclamate, acesulfame-K, aspartame and saccharin. High contents of cyclamate close to the maximum permitted level in 1999 of 400 mg/L were found in many soft drinks. The estimated intake of the sweeteners was calculated using the Danish Dietary Survey based on 3098 persons aged 1-80 years. The estimated intake with 90<sup>th</sup> percentiles of 0.7, 4.0 and 0.2 mg/kg bw/day for acesulfame-K, aspartame and saccharin respectively was much lower than the ADI values of 15 mg/kg bw/day, 40 mg/kg bw/day and 2.5 mg/kg bw/day for acesulfame-K, aspartame and saccharin respectively. However, the 90<sup>th</sup> percentile of the estimated cyclamate intake in 1-3 year olds was close to the ADI value of 7 mg/kg bw/day and the 99<sup>th</sup> percentile in the 1-10 year olds far exceeded the ADI value. Boys aged 7-10 year had a significantly higher estimated intake of cyclamate than girls. The 90<sup>th</sup> percentile for the whole population was 1.8 mg/kg bw/day. After the reduction in the maximum permitted level in EEC in 2004 from 400 to 250 mg cyclamate/L the exposure in Denmark can be expected to be reduced. A new investigation in 2007 should demonstrate whether the problem with high cyclamate intake is now solved.

**Keywords**

Estimated intake, intense sweeteners, Denmark, cyclamate, acesulfame-K, aspartame, saccharin, children.

## Introduction

Artificial sweeteners have been in use for many years for their sweet taste with less calories (Corti 1999). Due to increasing problems with obesity in developed countries and a general growing awareness of health implications connected to a high calorie diet, the use of sweeteners is increasing (Fry 1999). The worldwide increase in the number of people suffering from diabetes also contributes to an increased use of artificial sweeteners, as they have higher intake than non-diabetics (Food Standards Australia and New Zealand 2004). Finally sweeteners reduce tooth decay.

The food safety of the consumer needs to be assured. Thus in 1994 and 1995 the European Commission announced three directives, regarding sweeteners, colours and miscellaneous additives, setting the maximum permitted levels (MPL) with later adjustments. The directives also demand that all member states report intake of food additives, including sweeteners every year. The Commission is advised by the European Food Safety Authority (EFSA). EFSA is allocating the Acceptable Daily Intake (ADI) of all approved food additives, which is an intake that can be ingested daily over a lifetime without appreciable health risk. The MPL are adjusted to avoid consumer intake in excess of the ADI.

The ADI of sweeteners are 40 mg/kg body weight/day for aspartame, 7 mg/kg bw/day for cyclamate, 5 mg/kg bw/day for saccharin and 15 mg/kg bw/day for acesulfame-K.

The Danish Dietary Survey (Andersen et al. 1996) provides extensive information on the intake of foodstuffs in a representative part of the population aged 1 to 80 years. The food survey can provide general information on what the Danes eat and in which quantities. An

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4 estimation of the intake of specific compounds is possible if the level of the compound in  
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6 all potential foodstuffs is known.  
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11 The Danish monitoring programme for food additives samples foodstuffs for chemical  
12 analysis. The primary objective is to check that the additives are used legally and within  
13 the maximum permitted levels, but the analyses are also utilized to survey the intake of  
14 food additives. Non-alcoholic beverages are the major source of the intake of intense  
15 sweeteners (Bergsten 1998) .  
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26 In a recent review of intake estimations of intense sweeteners (Renwick 2006) it appears  
27 that very few intake estimations based on large representative parts of the population have  
28 been carried out and only one for people under 12 years of age. Thus, it was the purpose  
29 of this investigation to determine the intake of intense sweeteners with non-alcoholic  
30 beverages in different age groups of the Danish population.  
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## 40 **Materials and methods**

### 41 *Monitoring program*

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43 In 1999 the project: "Monitoring of additives: Intense sweetening agents in non-alcoholic  
44 beverages" was carried out as part of the The Danish Veterinary and Food  
45 Administration's programme for monitoring of additives (Andersen and Okholm 2001). An  
46 initial market survey of non-alcoholic beverages was carried out to establish the number of  
47 products relevant for analysis. 246 products were found on the market. One unit of each  
48 product was bought over a 3-month period in the autumn of 1998 by local authorities from  
49 the 12 largest supermarket chains all over Denmark covering 81% of the market and the  
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4 samples were stored in refrigerator at 5°C. 116 samples were chosen for chemical  
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6 analysis for sweetening agents. All the samples declared to contain the sweeteners were  
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8 selected. Besides, minimum one variant of taste from the remaining groups of samples  
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10 was selected for comparison and checking for undeclared use of intense sweeteners. Of  
11  
12 the 116 samples analysed, 62 were soft drinks without carbon dioxide (50 light and 12  
13  
14 sugar sweetened), 36 were soft drinks with carbon dioxide (21 light and 15 sugar  
15  
16 sweetened) and 18 were fruit juices (10 light and 8 sugar sweetened or not sweetened at  
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18 all). By “light” is understood soft drinks with a declared content of artificial sweeteners. 109  
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20 samples were of Danish origin and seven were imported.  
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### 28 *Chemical analysis*

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30 For acesulfame K, aspartame and saccharin the beverages were diluted if necessary with  
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32 deionised water to a suitable concentration level of acesulfame-K, aspartame and  
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34 saccharin. The sweeteners were separated by HPLC on a C-18 column, 5 µm, 250 x 4.6  
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36 mm equipped with a guard column, eluted isocratically with a mixture of methanol and  
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38 water buffered with potassium hydrogen phosphate and measured spectrophotometrically  
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40 at 220 nm. The contents were calculated from standard curves with six standard  
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42 concentration levels (external standardisation). For cyclamate the beverages were diluted  
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44 if necessary with deionised water to a suitable concentration level of cyclamate, which was  
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46 derivatised by shaking in a separating funnel with sulphuric acid, n-heptane and sodium  
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48 hypochlorite to N,N-dichlorocyclohexylamine. The heptane phase was separated, washed  
49  
50 with sodium carbonate and dried with sodium sulfate. The cyclamate was determined by  
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52 HPLC on a C-18 column, 5 µm, 250\*4.6 mm equipped with a guard column, eluted  
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54 isocratically with methanol and water and measured spectrophotometrically at 314 nm.  
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4 The content was calculated from standard curves with six standard concentration levels  
5 also derivatised like the samples (external standardisation). All chemicals were analytical  
6 grade and the solvents were HPLC grade. The laboratory was accredited to perform the  
7 methods according to ISO 17025.  
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16 In every series of samples was included a duplicate divided between two series and a  
17 recovery experiment with addition of the sweeteners to the original sample in the same  
18 amount as judged to be in the sample. From the 30 duplicate determinations the relative  
19 internal reproducibilities were calculated to be 2.2%, 2.5%, 2.9% and 1.9% for acesulfame-  
20 K, aspartame, cyclamate and saccharin, respectively. From the 15 recovery experiments  
21 recoveries of 100%, 103%, 98% and 100% for acesulfame-K, aspartame, cyclamate and  
22 saccharin, were calculated respectively. The results showed that the analytical methods  
23 had been under control at all times with no values exceeding the control limits for double  
24 determinations or for recoveries.  
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#### 40 *Dietary survey*

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42 The survey was conducted in 1995 on a random sample of Danes 1-80 yrs drawn from the  
43 Central Population Register, stratified by sex and age (Andersen et al. 1996). Dietary data  
44 were based on a 7-day pre-coded record method with closed answering categories  
45 supplemented with possibility for open answers. Participants in the survey kept the record  
46 for 7 consecutive days. The questionnaire was designed to cover the whole diet. A  
47 combination of food quantification methods (household measures and estimation from  
48 photos of different portion sizes) were used to get the best estimate of amounts  
49 consumed. For beverages household measures (e.g. cups, glasses, bottles) were used.  
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4 The questionnaire covered 207 food items, including 20 beverages (e.g. different types of  
5 milk, beer, wine, soft drinks and fruit juices). Sweetened beverages included two types of  
6 beverages with carbon dioxide: soft drinks light and soft drinks sugar sweetened and two  
7 types of beverages without carbon dioxide: soft drinks sugar sweetened and soft drinks  
8 light including fruit juices.  
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18 The food record was validated against urinary nitrogen as part of a pilot study (Groth and  
19 Fagt 1997). In all 1261 children 1-14 yr and 1837 adults 15-80 yr participated in the  
20 survey. For children below 10 yr of age parents recorded the food intake for the child. For  
21 children aged 10-12 yr parents decided whether the child was capable of filling in the  
22 records or not. 39 adults reported that they suffered from diabetes, but no information  
23 about diabetes in the children was reported. However, the prevalence of diabetes among  
24 children is considered much lower than for adults.  
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### 35 36 37 *Estimated intake calculation*

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39 The 116 samples of beverages analysed were aggregated into four different types of  
40 beverages for which intake data was present: with carbon dioxide, light (average intake 24  
41 ml/day) and sugar sweetened (average intake 108 ml/day) and without carbon dioxide,  
42 light (average intake 71 ml/day) and sugar sweetened (average intake 136 ml/day). For  
43 each person the registered average daily intake of the four categories was multiplied with  
44 the content of intense sweetener, and a calculation of basic statistic parameters of the  
45 intake distribution was carried out in the form of mean, standard error of mean and  
46 percentiles with the persons divided in age groups, males, females and the total  
47 population. For the content of intense sweetener was used two scenarios: One with the  
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4 analytical determined mean value for all the light samples with content of the specific  
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6 sweetener called “high intake” and one with the analytical determined mean value for all  
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8 the light samples in the category called “average intake”, which will be lower than “high  
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10 intake”, since many of the light samples were not sweetened with all the four artificial  
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12 sweeteners. The variability in the levels of sweeteners inside the four categories of soft  
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14 drinks has not been used in the estimated intake calculation.  
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## 21 Results

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23 The maximum permitted levels (MPL) for the sweeteners were at the time of this study 350  
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25 mg acesulfame-K/L, 600 mg aspartame/L, 400 mg cyclamate/L and 80 mg saccharin/L.  
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28 Table I shows the distribution of the sweeteners in the different types of beverages with  
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30 average for all the samples in the aggregated four categories of beverages used for an  
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32 average intake calculation. Table II shows the average, range and number of samples with  
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34 content of the specific sweetener used for a high intake calculation.  
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37 [Insert table I and II about here]  
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42 No illegal use of the four examined sweetening agents was found in the analysis of 62  
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44 different products of soft drinks without carbon dioxide. One soft drink exceeding the  
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46 maximum permitted level for cyclamate was found (454 mg/L). 13 faulty declarations were  
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48 found in 12 samples, most often a sweetener found which was not declared, amongst  
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50 others, three samples of Ice Tea with added aspartame, and a sample of a sugar  
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52 sweetened product with a small content of aspartame.  
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4 No illegal use of the four examined sweetening agents was found in the analysis of 18  
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6 different products of juice. The maximum permitted level was not exceeded in any product.  
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9 Six faulty declarations were detected in three samples (more than one sweetener per  
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11 sample not declared). One sugar sweetened product also contained acesulfame-K and  
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13 aspartame. The sum total of the 62 samples of soft drinks and the 18 juice samples equals  
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15 the 80 samples in the category of beverage without carbon dioxide.  
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19 No illegal use of the four examined sweetening agents was found in the analysis of 36  
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21 different products of soft drinks with carbon dioxide. The maximum permitted level was not  
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23 exceeded in any product. Two faulty declarations were detected in two samples. One  
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25 sugar-sweetened product also contained a high amount of aspartame.  
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30 The sum of all the intense sweeteners in the products is shown in Figure 1 and 2 for soft  
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32 drinks without carbon dioxide (including juice) and with carbon dioxide respectively and  
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34 where beverages without intense sweeteners are excluded from the figures. It appears  
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36 that if added at all, the level of cyclamate is often very close to the maximum permitted  
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38 level. For the other sweeteners the content is usually much lower than the maximum  
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40 permitted levels.  
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47 [Insert fig. 2 and 3 about here]  
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52 In the present study representative for the Danish market, most often a mixture of  
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54 sweeteners is used with cyclamate as the dominating sweetener, especially in beverages  
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56 without carbon dioxide. In soft drinks with carbon dioxide a mixture of aspartame and  
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58 acesulfame-K is more often used to sweeten the products. Two intake calculations were  
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4 carried out. One using an average only of the samples with a content of sweetener called  
5 high intake (Figure 3), and another with an average of all samples in each of the four  
6 categories of beverages called average intake (Figure 4). Estimated intake in mg/kg  
7 bw/day was calculated from an average for each age group of the actual body weights  
8 registered during the food survey.  
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18 [Insert Fig. 3 and 4 about here]  
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23 Both high intake and average intake of all the sweeteners for all persons are shown in  
24 Table III for four of the age groups of the intake distribution and for boys and girls in one  
25 age group.  
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32 [Insert Table III about here].  
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### 36 37 **Discussion**

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39 To take into account brand loyalty (Leclercq et al. 2003) we have found it relevant to carry  
40 out a high intake calculation by averaging only the samples with the analytical determined  
41 content of intense sweeteners. Even higher estimated intakes could be found under the  
42 assumption that a person only drinks a beverage with the highest found content of intense  
43 sweetener, but that would be a different brand for each of the four sweeteners and  
44 therefore not a very likely scenario. However, it could also be argued that the average for  
45 all samples in each of the four categories of beverages is a more reasonable figure to put  
46 into the intake calculation, so that extremes do not dominate the intake calculation.  
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59 Therefore this calculation has also been carried out.  
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7 The results for cyclamate are presented in Figure 3 and for all the sweeteners in Table III.  
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9 For cyclamate the differences between the high and average intake are not so great,  
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11 because most of the soft drinks both with and without CO<sub>2</sub> contained cyclamate and none  
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13 of the sugar sweetened drinks contained cyclamate (Table I and II). For aspartame the  
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15 differences are much greater (Table III) due to the fact that a few sugar sweetened  
16  
17 beverages also contained aspartame. Therefore just two samples increase the intake of  
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19 aspartame significantly, since they get to represent the whole category of sugar  
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21 sweetened soft drinks. This shows very well the limitations of the high intake calculation,  
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23 but since intake of acesulfame-K, aspartame and saccharin under all circumstances are  
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25 much lower than the ADI values, further refinement of the intake calculation is not  
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27 warranted.  
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35 However, the estimated cyclamate intake is much higher than the ADI value for groups of  
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37 children. For the high intake calculation (Figure 3) the 90<sup>th</sup> percentile in the cyclamate  
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39 intake distribution for 1–3 year olds of 7.1 mg/kg bw/day is close to the ADI value, and for  
40  
41 1-10 year olds the 99<sup>th</sup> percentile is much higher than the ADI value, and more than double  
42  
43 the ADI value for the group 1-3 year with 14.3 mg/kg bw/day (Table III), and the child with  
44  
45 the highest intake gets 18 mg cyclamate/kg bw/day. Boys have a significantly higher  
46  
47 estimated intake of cyclamate than girls in the age group 7-10 yr both on the 90<sup>th</sup> and 99<sup>th</sup>  
48  
49 percentile but not on the median value (Table III), and also for boys in the age group 11-14  
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51 yr the 99<sup>th</sup> percentile is higher than the ADI value The average intake calculations in Figure  
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53 4 give a slightly lower intake, but the 99<sup>th</sup> percentiles from 1-10 yr are still much higher  
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55 than the ADI value of 7 mg/kg bw/day, i.e. 13.1 mg for the age group 1-3 yr, and the child  
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4 with the highest intake gets 17 mg/kg bw/day. Apparently small children have a much  
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6 higher risk of high cyclamate intakes with their low body weights and the often reported  
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8 widespread use of dilutable artificial sweetened soft drinks in homes, kindergartens as  
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10 well as in day nurseries. It seems also that boys at a certain age have a higher cyclamate  
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12 intake than girls perhaps due to a higher activity level and therefore a higher consumption  
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14 of soft drinks.  
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21 This means that with a maximum permitted level of 400 mg cyclamate/L relatively large  
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23 groups of Danish healthy children had an intake of cyclamate higher than the new ADI  
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25 value of 0-7 mg/kg bw/day established in March 2000 by the revised opinion on cyclamic  
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27 acid and its sodium and calcium salts from the Scientific Committee on Food (SCF 2000).  
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29 Therefore Denmark presented these data for the EEC Commission in 2001 and argued for  
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31 a lower permitted level of cyclamate in beverages, and in January 2004 the permitted level  
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33 was reduced from 400 mg to 250 mg cyclamate/L. However, FAO/WHO is still  
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35 recommending a maximum level of 400 mg/L and also the high ADI value of 11 mg/kg  
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37 bw/day (Codex 2006).  
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45 Most studies dealing with intake of sweeteners have focused on high consuming  
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47 subgroups e.g. diabetes patients or teenagers (Baer A. and Biermann C. 1992; Arcella et  
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49 al. 2004; Garnier-Sagne et al. 2001; Ilback et al. 2003), and cyclamate intakes above the  
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51 ADI value is found in some cases, especially for children. The calculation of intake of  
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53 sweeteners based on analytical determined contents of all brands on the market and in a  
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55 large representative part of the population is well suited to identify high consuming groups  
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57 in the general population. As seen in this investigation children may be high consumers,  
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4 even though they are not diabetics. Very few other similar investigations on a large  
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6 representative part of the population exist. However, an investigation from Australia and  
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8 New Zealand (Food Standards Australia and New Zealand 2004) showed a 95<sup>th</sup> percentile  
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10 for cyclamate intake in a high consumer group aged 12 to 39 years of 9.3 mg/kg bw/day  
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12 and in a group of diabetics of 11.6 mg/kg bw/day, which exceed the EEC ADI value of 7  
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14 mg/kg bw/day. Furthermore, the Food Standards Agency in UK (Food Standards Agency  
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16 2003) has conducted an investigation of the intake of aspartame, acesulfame-K, saccharin  
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18 and cyclamate in soft drinks with similar conclusions as in the present investigation. High  
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20 consumers (97.5<sup>th</sup> percentile) from 1½ to 4½ years old had an intake of 14 mg  
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22 cyclamate/kg bw/day apparently calculated as the high intake calculation in the present  
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24 investigation, i.e. an average only for the children, who consumed the sweeteners in  
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26 question. However, values for content of sweeteners were not determined analytically, but  
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28 based on information from the manufacturers. Based on these results FSA advised  
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30 parents to give young children no more than three beakers (about 180 ml each) a day of  
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32 dilutable soft drinks.  
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42 The other significant source of intense sweeteners is tabletop sweeteners. High  
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44 consumers of cyclamate based tabletop sweeteners ate 230 mg/day corresponding to  
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46 35% of the ADI (Bergsten 1998). However, tabletop sweeteners are typically not used by  
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48 healthy children, so they are without significance for the high cyclamate intake found in this  
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50 investigation in groups of small children.  
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56 Although from 1999 this investigation has still a value as the only investigation with  
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58 analytical data for all artificial sweetened soft drinks on the market and with a food survey  
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4 covering a large representative part of the population demonstrating a high estimated  
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6 intake of cyclamate in children. The intake of cyclamate in EEC countries could be  
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8 expected to have decreased after the reduction of the maximum permitted level in 2004  
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10 from 400 mg/L to 250 mg/L. However, Codex still recommends 400 mg/L and an ADI of 11  
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12 mg/kg bw/day, thus the cyclamate intake in the rest of the world probably remains high.  
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19 A new investigation of soft drinks on the Danish market will be started in 2007 with food  
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21 survey data from 2000 to 2005 to ascertain whether also a maximum permitted level of  
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23 250 mg cyclamate/L will lead to higher cyclamate intakes in children than the ADI value of  
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25 7 mg/kg bw/day.  
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### 30 **Conclusions**

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33 In an investigation from 1999 it was demonstrated that almost 10% of Danish children  
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35 aged from 1 to 10 years had an estimated cyclamate intake higher than the ADI value of 7  
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37 mg cyclamate/kg bw/day. Boys from 7-10 year had a significantly higher intake of  
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39 cyclamate than girls of the same age group. The intake of acesulfame-K, aspartame and  
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41 saccharin was far below their respective ADI-values. After the reduction in the maximum  
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43 permitted level in EEC in 2004 from 400 to 250 mg cyclamate/L the exposure in Denmark  
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45 and other EEC member states can be expected to be reduced. A new investigation in  
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47 2007 shall demonstrate whether the problem with high cyclamate intake is now solved.  
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7 **Table I.** Intense sweeteners in non-alcoholic beverages, average of all samples in each  
8 category (for average intake calculation)  
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11 **Table II.** Intense sweeteners in non-alcoholic beverages, average of samples with content  
12 of sweetener (for high intake calculation), (range) and (number of samples with content of  
13 sweetener)  
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16 Table III. Intake of intense sweeteners in the whole population and in children from 1-10  
17 years, mg/kg bw/day  
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19 **Figure 1.** Intense sweeteners in soft drinks without carbon dioxide  
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22 **Figure 2.** Intense sweeteners in soft drinks with carbon dioxide  
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24 **Figure 3.** High estimated intake of cyclamate from non-alcoholic beverages - All  
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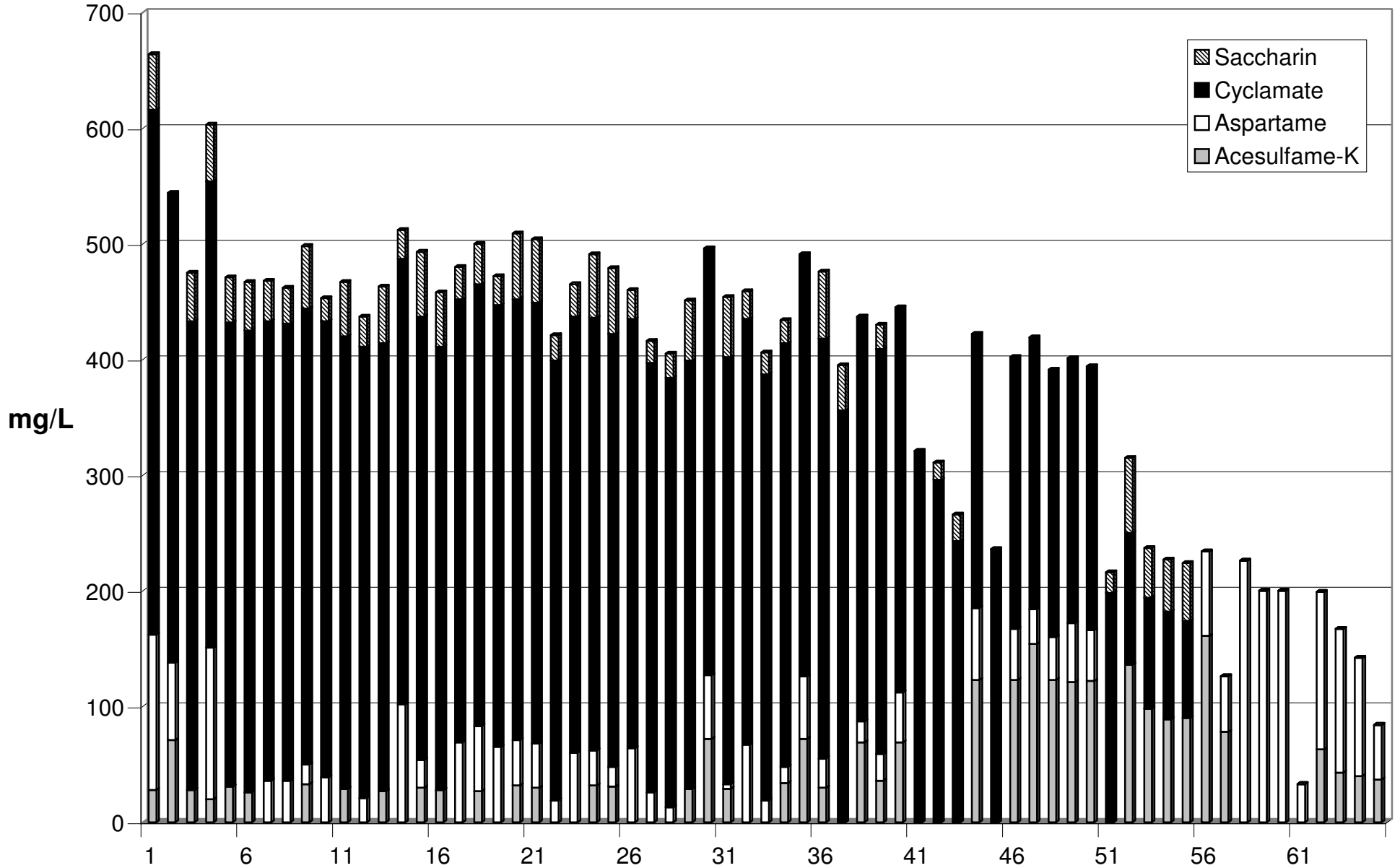
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27 **Figure 4.** Average estimated intake of cyclamate from non-alcoholic beverages – All  
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Type of sample	Number of samples	Acesulfame-K mg/L	Aspartame mg/L	Cyclamate mg/L	Saccharin mg/L
Soft drinks (with CO <sub>2</sub> ) Light	21	49	152	152	15
Soft drinks (with CO <sub>2</sub> ) With sugar	15	-	30	-	-
Soft drinks (no CO <sub>2</sub> ) Light	60	42	36	305	26
Soft drinks (no CO <sub>2</sub> ) With sugar	20	2	35	-	-

Type of sample	Acesulfame-K mg/L	Aspartame mg/L	Cyclamate mg/L	Saccharin mg/L
Soft drinks (with CO <sub>2</sub> ) Light	129 (32-179) (n=8)	159 (26-460) (n=20)	245 (203-404) (n=13)	29 (7-58) (n=11)
Soft drinks (with CO <sub>2</sub> ) With sugar	-	450 (n=1)	-	-
Soft drinks (no CO <sub>2</sub> ) Light	62 (20-161) (n=39)	56 (4-226) (n=43)	333 (84-454) (n=55)	37 (15-65) (n=42)
Soft drinks (no CO <sub>2</sub> ) With sugar	43 (n=1)	124 (n=1)	-	-

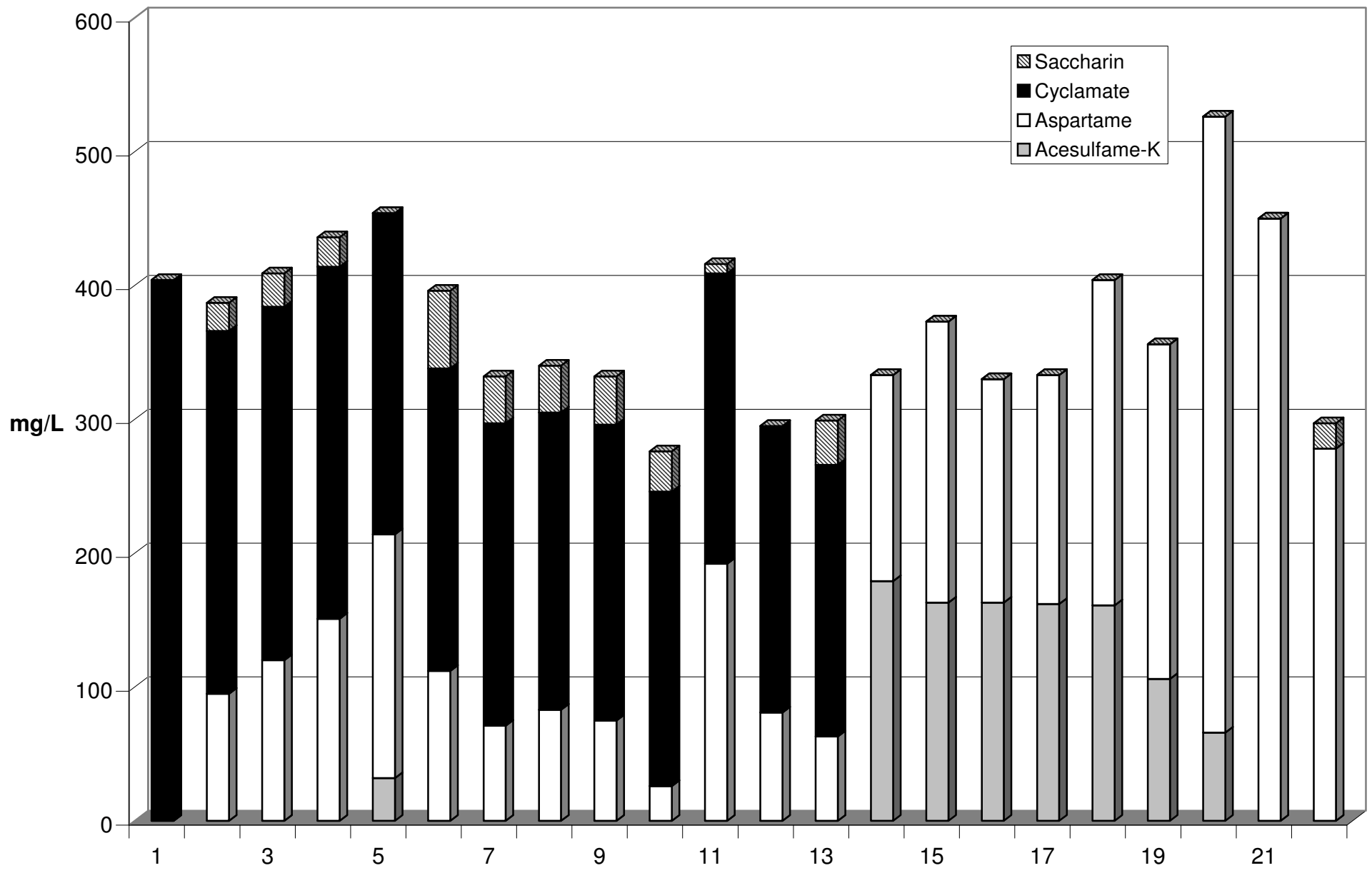
Sex	Age years	Percentile	Acesulfame-K		Aspartame		Cyclamate		Saccharin	
			Av. mg/kg bw/day	High	Av. mg/kg bw/day	High	Av. mg/kg bw/day	High	Av. mg/kg bw/day	High
All	1-80	50	0.1	0.1	0.1	0.8	0.1	0.1	0.01	0.01
		90	0.3	0.7	0.7	4.0	1.6	1.8	0.1	0.2
		99	0.9	2.0	1.8	9.4	6.1	6.9	0.5	0.8
All	1-3	50	0.3	1.0	0.8	3.7	1.6	1.8	0.1	0.2
		90	0.9	2.3	1.9	8.8	6.3	7.1	0.6	0.8
		99	1.9	3.9	2.8	16.9	13.1	14.3	1.1	1.6
All	4-6	50	0.2	0.8	0.7	3.4	1.1	1.2	0.1	0.1
		90	0.7	1.7	1.4	7.3	5.3	5.8	0.5	0.7
		99	1.6	3.7	2.6	12.4	10.2	11.7	0.9	1.3
Boys	7-10	50	0.2	0.6	0.7	3.8	1.1	1.2	0.1	0.1
		90	0.7	1.6	1.3	7.3	5.2*	5.8*	0.3	0.6
		99	1.5	3.1	2.5	13.0	10.5*	11.7*	0.5	1.3
Girls	7-10	50	0.2	0.6	0.6	3.4	0.9	1.0	0.1	0.1
		90	0.6	1.3	1.1	6.1	3.9*	4.2*	0.4	0.5
		99	1.0	2.5	1.9	9.7	6.1*	6.9*	0.7	0.8
ADI			15		40		7		2.5	

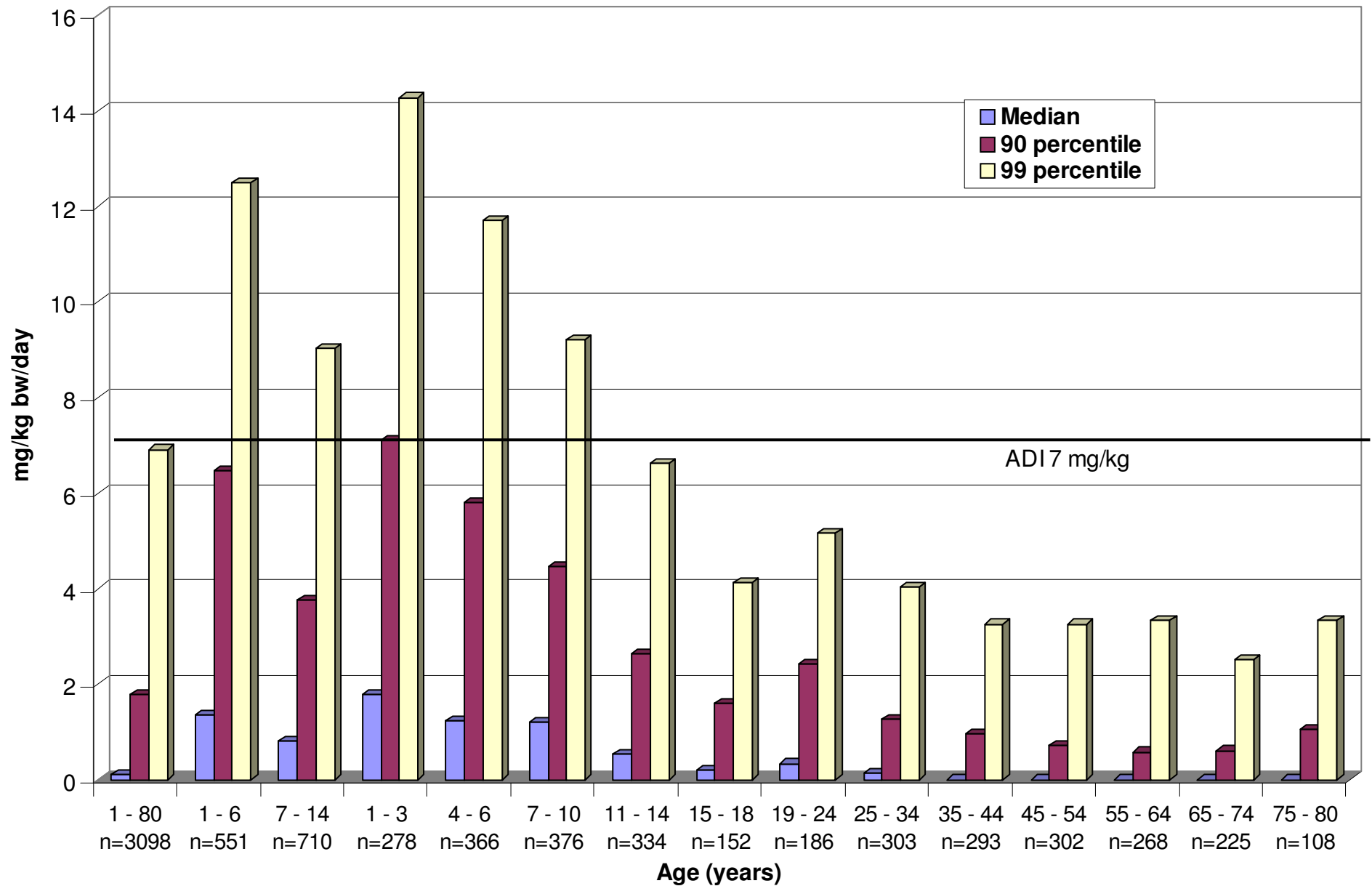
\* T-test shows significant difference between boys and girls on the 99.9% probability level



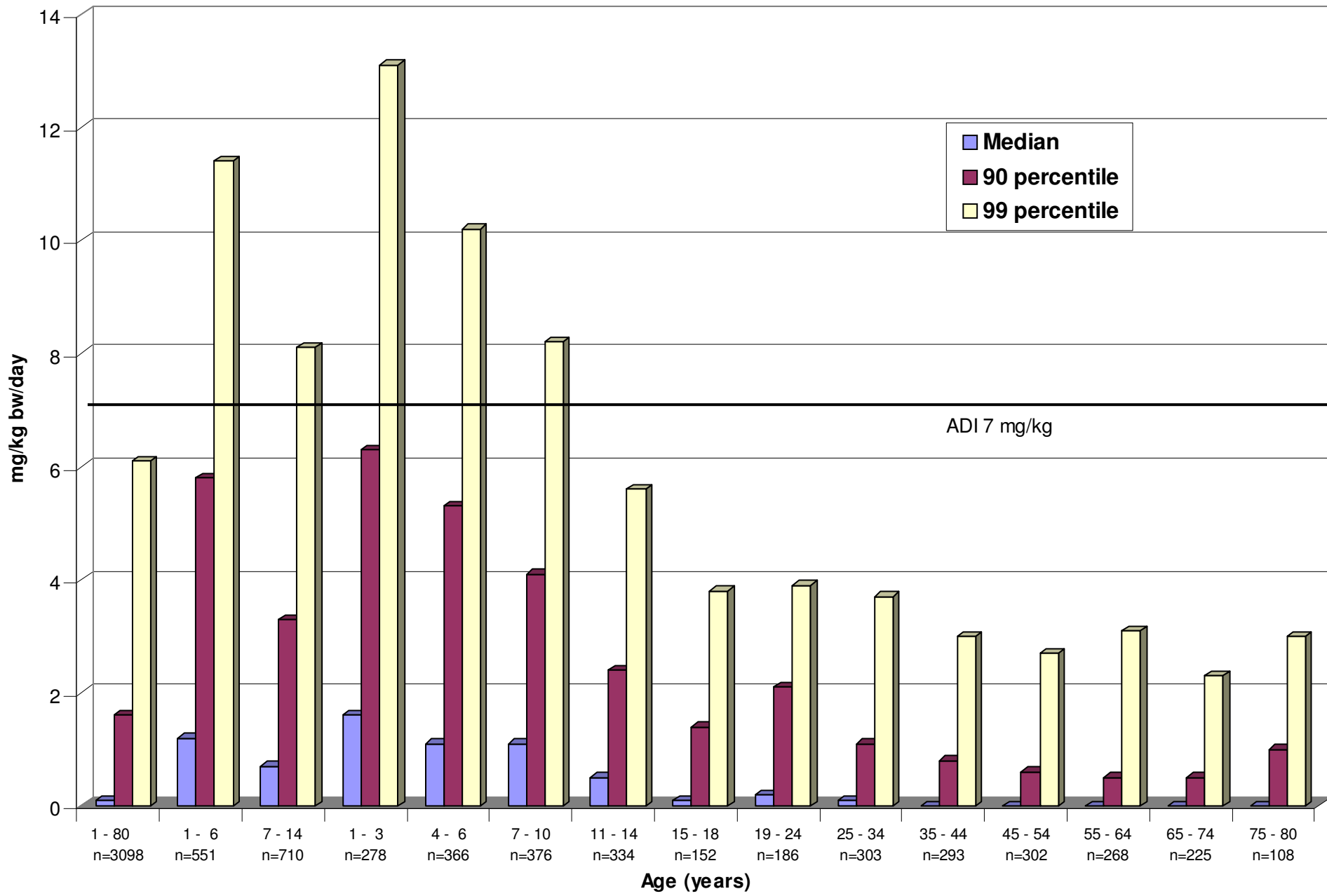


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