EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Directorate B - Scientific Health Opinions
Unit B3 - Management of scientific committees II

Scientific Committee on Food

SCF/CS/EDUL/192 final
13 March 2000

REVISED OPINION ON CYCLAMIC ACID AND ITS SODIUM AND CALCIUM SALTS

(Expressed on 9 March 2000)

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Revised opinion on cyclamic acid and its sodium and calcium salts

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Terms of reference

To review and revise the opinion on cyclamate, expressed on 14th December 1995, at the 99th SCF meeting, in the light of scientific information requested in that opinion, now made available.

This new information consists of the following:

- study on conversion rates in converters and non-converters;
- discussion of *in vitro* studies to compare testicular sensitivity;
- epidemiology studies: intake survey in Catalonia, fertility of workers involved in cyclamate manufacture, cyclamate intake and male fertility case control study.

Background

The Scientific Committee for Food (SCF) reviewed the toxicity of cyclamate, cyclohexylamine and dicyclohexylamine in 1985 and established a temporary ADI of 0-11 mg/kg bodyweight (bw), expressed as cyclamic acid, for cyclamic acid and its sodium and calcium salts (1). The ADI was based on a no-observed-adverse-effect-level (NOAEL) of 100 mg/kg bw in the rat for testicular toxicity of cyclohexylamine, the metabolite of cyclamate produced by microbial fermentation of unabsorbed cyclamate in the lower gut. At that time, the fraction of ingested cyclamate not absorbed was assumed to be 63% and the conversion rate of unabsorbed cyclamate to cyclohexylamine was estimated to be 30%, giving an overall conversion rate of 18.9%. The ADI was temporary because of uncertainties relating to the relevance for man of the testicular damage found in rats fed cyclohexylamine.

The Committee reviewed cyclamate again in 1988, 1991 and 1995 (2-4). The Committee was informed about studies confirming that cyclohexylamine is metabolised similarly by rat and man. Further information concerning the intra-individual variability in cyclamate conversion in humans with time and two studies in *Cynomolgus* monkeys on the testicular toxicity of cyclohexylamine were submitted by the petitioner. In 1995, preliminary data from three different epidemiology studies being performed in Spain were also available to the Committee.

The Committee agreed that these submissions from the petitioner did not answer its questions about the intra-individual variability of cyclamate conversion with time and the testicular toxicity of cyclohexylamine. In particular, the monkey studies did not provide any basis for concluding that primates are less sensitive than the rat to the effects of cyclohexylamine on the testis. Rather, they had provided evidence that the effects were of toxicological concern in primates as they were in all other species so far tested with the exception of the mouse. However, it was not possible to establish a clear NOAEL from the monkey studies. After considering all new data available, the Committee concluded in 1995 (4) that the uncertainties still existed and confirmed the NOAEL for cyclohexylamine in the rat of 100 mg/kg bw as the

basis for the ADI and the temporary ADI of 0-11 mg/kg bw for cyclamate was maintained, pending submission of further data as follows:

i) Given the consumption of cyclamate is likely to be on a regular basis among users, the following information is required on conversion rates in humans, using reasonable sample sizes, to be submitted within 2 years of publicising this opinion in the SCF minutes:
   a) to establish what proportion of initially low/negligible converters can become high converters over time with repeated exposure for a few weeks to cyclamate;
   b) for those who do convert, to establish within individuals the range of conversion rates which occur over time following repeated exposure to cyclamate, paying particular attention to inter-individual differences in the time course of induction and the persistence and magnitude of the capacity to convert in high converters.

ii) If feasible, *in vitro* studies to compare the relative sensitivity of human, monkey and rat testicular tissue to cyclohexylamine.

iii) The completed reports of three Spanish epidemiology studies.

In response to this request the petitioner submitted further information concerning conversion rates in human converters and non-converters, a review of the potential for *in vitro* investigation of the testicular toxicity of cyclohexylamine, and reports of the two Spanish epidemiology studies comprising fertility of workers involved in cyclamate manufacture and a case-control study on cyclamate intake and male fertility. A report on intake survey in Catalonia was also submitted.

The Committee is also aware of a recent publication on long term toxicity and carcinogenicity of cyclamate in primates (5). The study was inconclusive and the Committee was unable to use it for risk assessment.

Intake surveys

The amount and type of all foods, drinks and drugs consumed in a random sample of 2450 subjects of the Catalan population, aged 6-75 years, were obtained by two 24-hour recalls at different seasons in 1992 (6). A food frequency questionnaire with 77 items was used and cyclamate intake was calculated from the reported intakes of foods and the sweetener content, obtained from the respective manufacturers. 18% of the population consumed cyclamate (21% in males, 15.4% in females), the highest percentage of 33% consumers was found in men aged 35-44 years. Average daily cyclamate intake was estimated to be 0.4 and 2.4 mg/kg bw in the whole population and in consumers, respectively. In subjects on a diabetic diet (3% of the population, no differentiation between consumers and non-consumers), the average consumption of cyclamate was estimated to be 1.6 mg/kg bw. Among all consumers, the highest intake of 4.9 and 4.4 mg/kg bw was estimated in males and females of the age group 6-17 years. Four subjects (0.16% of the population, 0.9% of consumers) were estimated to have a cyclamate intake exceeding the tADI (i.e. >11 mg/kg bw).

In an earlier study performed in Germany (7), 31% of the study population (2291 persons) consumed cyclamate and the mean daily intake was estimated to be 2.6 and 6.1 mg/kg bw among consumers and heavy consumers (90^{th} percentile of intake), respectively. Interestingly, persons who adhered to a diet (diabetes, weight control) did not ingest cyclamate in substantially higher amounts. Similar figures for daily intake of cyclamate for users and

persons on a diet were also reported from Brazil (8), while among 212 teenagers in Italy, cyclamate use seems to be low (6% users with a mean daily intake of 0.24 mg/kg bw) (9). In The Netherlands, 11% of the study population (6060 persons) consumed cyclamate and the median daily intake estimated from a two-day record was 1.1 and 4.9 mg/kg bw among consumers and heavy consumers (90^{th} percentile of intake), respectively (10). Other intake data on cyclamate were also available to the Committee. However these data were not included as intake estimates were not performed on a comparable basis.

Study on conversion rates in converters and non-converters.

In order to obtain a reasonable sample size of converters of cyclamate, an initial screening study was performed with 261 subjects (125 males and 136 females) receiving the equivalent of 3x250 mg cyclamic acid per day as calcium cyclamate during one week. Nine males and 10 females were identified as converters, based on urinary excretion of cyclohexylamine (>0.5% metabolism of cyclamate to cyclohexylamine). 7 males and 7 females were selected and agreed to participate in the main study as well as 31 non-converters (<0.3% metabolism, 19 males and 12 females), based on urinary excretion of cyclohexylamine.

In the main study, the metabolism of cyclamate to cyclohexylamine was studied for 13 weeks in the 45 subjects selected. The daily dose of cyclamic acid was again 3x250 mg, given in tablets as calcium cyclamate. A controlled, timed 3-hour urine collection was performed twice per week on weeks 1-3 and 7-13 and on the remaining study days, 20ml urine specimens were collected. Blood samples were collected once weekly at the time of the 3-hour urine collection. All samples were analyzed for cyclohexylamine.

Of the 31 non-converters, 30 essentially remained non-converters. One female subject showed a variable increase in the conversion rate, the highest rate was 1% on day 4. The average conversion rate during steady state in this subject was 0.24%. Of the 14 subjects selected as converters, one subject turned out to be essentially a non-converter (average conversion rate during steady state 0.03%), 4 showed consistent low average conversion rate of 3-4% in the steady state, 4 subjects showed an average conversion rate of 8-20% and 5 subjects converted on the average between 25 and 46% of the cyclamate to cyclohexylamine. Large intra-individual variations were observed in all converters. The highest conversions observed on any single day in the 5 subjects with the highest average conversion rate range from 57-85%. The individual variation can be illustrated by the highest converter (85,4% on day 60) who converted 75% on day 49, 6.5% at day 56 and 42.7% at day 67; the average conversion rate over days 7-91 was 26%. In 5 subjects, a tendency towards an increased conversion rate over time was seen, reaching an average conversion rate during steady state of >18.9% and one subject showing a constant average conversion rate of 36%. In the remaining 7 subjects average conversion rate was <18.9% and no increase of the conversion rate over time was observed. Plasma cyclohexylamine levels in converters were consistent with urinary data. A high renal clearance of 447±127 ml/min (7.2±2.1 ml/min/kg bw) was found.

In conclusion, none of those initially non-converters became a high converter following repeated exposure to cyclamate for 90 days. In the 13 converters studied, large intra-individual daily variations were seen and in 6 subjects average conversion rate exceeded 18.9% by a factor of up to 2.5.

Discussion of *in vitro* studies to compare testicular sensitivity.

No *in vitro* studies to compare the relative sensitivity of human, monkey and rat testicular tissue to cyclohexylamine were performed. Instead, a review of the available data in relation to the potential use of *in vitro* testicular systems to study species sensitivity, as suggested by the Committee, was submitted.

The testis is a complex tissue with many cell types and multiple interactions between the various types. It is not possible to model the whole of the spermatic cycle by *in vitro* systems, limiting the value of testis-derived *in vitro* systems for investigating species comparisons of toxicity, in the absence of knowledge on the target cell population, or the mechanism of toxicity of a compound. The review concluded that target cells for testicular toxicity of cyclohexylamine in the rat are most likely the Sertoli cells but target cells in dog and monkey testes have not been identified. Therefore, an appropriate *in vitro* system should include both (primary) Sertoli and germ cells. However, the relative *in vitro/in vivo* sensitivity of animal species has not been established and no inter-laboratory validation studies have been performed with such *in vitro* systems. Furthermore, no appropriate culture system for comparing rat, monkey and human cells has been studied systematically. Although, in principle, it could be possible to establish primary cell cultures of human Sertoli and germ cells, the cells to be used are likely to be inappropriate for studying the sensitivity of the developing testes to cyclohexylamine as the provenance of the cells would probably be from elderly patients undergoing surgery due to prostatic cancer and healthy human tissue would not be available.

In view of these major questions with respect to the reliability, reproducibility, and relevance of any data on the sensitivity of animal and human testicular cells to cyclohexylamine, *in vitro* studies for quantitative comparisons of the sensitivity of animal and human tissue were considered to be not feasible.

Epidemiology studies: fertility of workers involved in cyclamate manufacture

A total of 18 workers involved in the manufacture of cyclamate were studied, 4 of them having a high, direct exposure to cyclohexylamine for about 20 years and 1 had a high direct exposure to cyclohexylamine for 2 years. Reproductive history, a semen sample and a 24-hour urine specimen were provided by the workers. Because the workers were exposed to other situations which could have affected fertility, such as elevated working temperature, high alcohol consumption and smoking, the results are reported as a case study and no statistical analysis could be performed. In the whole group, only 1 worker had a normal sperm count and normal sperm motility according to the WHO criteria (11). The 5 workers with high direct cyclohexylamine exposure showed similar sperm counts, sperm motility and reproductive performance to other workers without direct cyclohexylamine exposure. Similarly, 10 workers with high measured urinary excretion of cyclohexylamine (0.12-1.45 mg/kg/day) were comparable to workers with a lower measured excretion of cyclohexylamine. However, as the 18 workers form a seemingly abnormal group both with respect to their low sperm count and their low sperm motility, the apparent lack of any influence of cyclohexylamine in the 4 exposed workers on these parameters is difficult to interpret and may not be extrapolated to the general healthy population. The Committee concluded that this study is of little significance for the safety evaluation of cyclamate.

Epidemiology studies: cyclamate intake and male fertility case control study.

Four hundred and five subjects, aged 30-50 years, attending a male infertility clinic in Barcelona because of >12 months' infertility were compared with 379 control subjects referred to the same clinic for a vasectomy. Two semen samples and a 24-hour urine specimen were provided by all subjects and the cyclamate intake was estimated from a semi-quantitative food frequency questionnaire between 1994 and 1996. There was no statistically significant difference in the estimated cyclamate intake between cases (0.72 mg/kg bw/day) and controls (0.55 mg/kg/day) or in the urinary cyclamate excretion (0.19 and 0.22 mg/kg bw/day in cases and controls, respectively). Cyclohexylamine was detected in the urine of 13% of cases and 12% of controls and the average values for urinary cyclohexylamine excretion in cases and controls (0.035 and 0.053 mg/kg bw/day) were not statistically significantly different.

Conclusion

The new epidemiological data revealed no indications of harmful effects on human reproduction parameters of either cyclamate used as food additive or of workplace exposure to cyclohexylamine, though the latter study was considered difficult to interpret.

The Committee noted that no *in vitro* studies to compare the relative sensitivity of human, monkey and rat testicular tissue to cyclohexylamine were performed but acknowledged the difficulties in performing such studies. The Committee now no longer requires such studies.

After considering all the data available on conversion of cyclamate to cyclohexylamine in humans, including the new data provided, the Committee concluded that the uncertainties with respect to the conversion rate in humans could be eliminated but that the 18.9% conversion rate used for establishing the temporary ADI of 0-11 mg/kg bw is no longer appropriate. There are large inter-individual variations observed in conversion rates and a lack of knowledge about the minimal time span of exposure to cyclohexylamine that might result in testicular damage. The Committee therefore concluded that the maximum observed individual overall conversion of cyclamate to cyclohexylamine and absorption of the latter would be 85%. This would be more appropriate for calculating an ADI. Since a maximum conversion figure would be utilised, a reduced safety factor should be applied for inter-individual differences (see below).

The Committee concluded that a full ADI for cyclamate could now be established. Taking a NOAEL of 100 mg/kg bw for cyclohexylamine, allowing for the difference in molecular weight between cyclamic acid and cyclohexylamine, using an 85% overall conversion rate for ingested cyclamate, and applying a 32-fold safety factor (see Annex), a full ADI of 0-7 mg/kg bw, expressed as cyclamic acid, for cyclamic acid and its sodium and calcium salts was established by the Committee.

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Annex: Derivation of ADI

Maximum overall conversion of cyclamate to cyclohexylamine and absorption of the latter is 85%.

NOAEL for cyclohexylamine is 100 mg/kg bw.

Allowance for difference in molecular weights between cyclamic acid and cyclohexylamine:

$$\frac{\text{MW cyclamate}}{\text{MW cyclohexylamine}} = 1.81, \text{ rounded up to } 2$$

safety factor[1]: 10 for inter-species extrapolation.
3.2 for inter-individual variations in toxicodynamics.
1 for inter-individual variations in toxicokinetics.

$$\text{ADI} = \frac{\text{NOAEL for CHA x MW ratio}}{\text{Safety factor x Conversion rate}} = \frac{100 \text{ x } 2 \text{ x } 100}{32 \text{ x } 85} = 7.35, \text{ rounded down to } 7 \text{ mg/kg bw}$$

[1] The safety factor of 100 nominally consists of a factor of 10 for inter-species extrapolation and a factor of 10 for inter-individual variations. According to the WHO (12), the factor of 10 for inter-species extrapolation can be subdivided into a a factor of 4 for toxicokinetics and a factor of 2.5 for toxicodynamics. The factor of 10 for inter-individual variations can be subdivided into 3.2 for toxicokinetics and 3.2 for toxicodynamics. As the highest individual conversion rate on a single day is used for calculating the ADI, no additional safety factor for inter-individual variations in toxicokinetics is needed.