European Food Safety Authority

Brussels, 1 October 2003
EFSA/AFC/FCM/17-final

Statement of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food updating the advice available on semicarbazide in packaged foods

Adopted on 1 October 2003

Statement of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food

updating the advice available on semicarbazide in packaged foods

Adopted on 1 October 2003

On 28 July 2003, EFSA issued preliminary advice on the possible occurrence of semicarbazide in certain packaged foods (available on the Internet at *http://www.efsa.eu.int/pdf/p_afc_doc_01.pdf*). The foods concerned are those packaged in glass jars and bottles closed with metal lids sealed with plastic gaskets that are foamed using the chemical blowing agent, azodicarbonamide. The Panel received new information on semicarbazide. The information included oral reports of the progress that has been made on analytical and technological aspects, oral presentation of the results of the genotoxicity studies commissioned by EFSA, a written report of a genotoxicity test conducted by industry, and some preliminary estimates of exposure. On the basis of this new information, the Panel offers the following further advice.

Analytical aspects

The current information indicates that semicarbazide detected in foods is not an artefact of the analytical method used to test foods (CIAA, 2003). The method, used so far utilises acid hydrolysis of the sample, derivatisation with 2-nitrobenzaldehyde (2NBA) and determination by LC-MS (liquid chromatography coupled to mass spectrometry) and it measures both free and bound semicarbazide. A new approach suitable for the direct analysis of free semicarbazide has been developed and applied. This has not yet been

validated in an inter-laboratory comparison but it has been used on different samples by three different laboratories. Using this new direct method, free semicarbazide has been found in heated samples of the blowing agent azodicarbonamide, in heated gasket formulations, and in aqueous extracts of gaskets (CIAA, 2003). The levels found are consistent with levels reported using the original analytical method for semicarbazide. On the basis of the available evidence, it can be concluded that free semicarbazide is formed as a consequence of the thermal treatment of azodicarbonamide, it is present in gaskets foamed using azodicarbonamide, and it migrates from these gaskets into foods.

The fate of semicarbazide in the packaged foods is unclear. The direct method of analysis is not yet developed sufficiently to test for free semicarbazide in foods. From basic organic chemistry, semicarbazide is known to react with chemicals such as carbonyl compounds and these functional groups are present in foods. Some or all of the semicarbazide in foods may therefore be bound and not free. It is known, for example, that in animal tissues exposed to nitrofurans antibiotics, where semicarbazide can be detected as one of the breakdown products of nitrofurans, it is not free semicarbazide but bound residues that are present. This binding is reversible, however, and this is the reason for using an acid hydrolysis step in the 2NBA method, in order to free the bound residues. Therefore, it is a reasonable assumption that the 2NBA method of testing foods is a worst case as it should determine both free and reversibly-bound residues of semicarbazide.

Exposure

Concentrations in food

There are few documented data on concentrations of semicarbazide in foods packed in glass jars and bottles. The Panel has been informed that industry intends to set up a database on semicarbazide concentrations in foods but this is not yet available. The foods that have been reported to contain semicarbazide include baby foods, fruit juices, jams and conserves, honey, ketchups and mayonnaise, pickles and sterilized vegetables and sauces. The levels of semicarbazide reported in these foods are variable, in the range non-detectable (less than 1 ppb; parts per billion, microgrammes/kg of food) up to 25 ppb. Baby foods are reported to have the higher concentrations, perhaps because of the higher ratio of gasket area to food mass for these small pack sizes.

Exposure estimates

The Panel has been informed that industry is developing estimates of exposure, based on actual consumption statistics and concentration data across Europe, and that these estimates should become available during October. In the interim, a preliminary estimation of the potential exposure to semicarbazide from food in glass jars and bottles has been made by the Panel. The estimate focused on babies because baby foods seem to contain the highest amounts of semicarbazide and because their food consumption is higher, on a body weight basis, than that of other age groups.

Intake of baby foods increases during weaning when milk is progressively replaced by solid food and then decreases after the first year of life when baby foods are progressively replaced by food not specifically intended for infants. The highest intake of the affected

foods is therefore likely to occur within the first year of life. This is confirmed by limited data from an Italian nationwide food consumption survey (Turrini, 2001). A 6-month-old baby fed only on processed baby foods was therefore taken to represent the worst case in terms of food consumption/kg body weight. On the basis of a limited inspection of food consumption patterns suggested for babies of 6 months, an intake of 700 g of such foods was assumed. Assuming a body weight of 7.5kg for a baby aged 6 months (CEC, 1993), and taking the highest concentration of semicarbazide reported of 25 ppb, the potential intake of semicarbazide would be 2.3 microgrammes/kg body weight/day. This estimation contains two conservative assumptions. First, exclusive recourse to commercial baby foods packaged in glass jars and bottles, an hypothesis that is not unrealistic. Second, that all the food consumed contains the highest level of semicarbazide reported.

Based on the types of food packaged in glass jars and bottles, in which semicarbazide has been found, it can be concluded that its estimated intake by adults and children on a bodyweight basis will be many times lower than the preliminary estimate of the intake for babies.

Toxicology

The main concerns about semicarbazide are its potential genotoxicity and carcinogenicity. The toxicological data available at present remain very limited.

Genotoxicity

In the new *in vitro* mutagenicity tests commissioned by EFSA, semicarbazide showed weak mutagenic activity mainly in the absence of a metabolic activating system. Positive

results were obtained in Salmonella Typhimurium strains detecting base pair substitutions (TA1535 and TA100) and in the mouse lymphoma *tk* forward mutation system (TNO, 2003). The other new study showed weak mutagenic activity only in Salmonella Typhimurium strain TA 1535 in the absence but not in the presence of a metabolic activating system (Industry study, 2003). The available results from an ongoing clastogenicity study in cultured mammalian cells are inconclusive and a second trial is being conducted (TNO, 2003). *In vivo*, in an earlier study on a series of hydrazine derivatives, the intraperitoneal administration of semicarbazide did not induce DNA damage, as measured by alkaline elution, in liver and lung tissue of mice, whereas positive results were obtained with chemically related genotoxic compounds (Parodi et al., 1981).

From these results it is apparent that semicarbazide is weakly genotoxic in some test systems *in vitro*. The limited *in vivo* experimental data available are insufficient to assess whether the activity observed *in vitro* is also expressed *in vivo*. Under these circumstances, it would be usual to call for *in vivo* genotoxicity tests to be conducted. However, the Panel is of the view that such studies, conducted according to standard protocols, may be inconclusive. The Panel considers that a better approach would be to conduct toxicokinetic studies to find out whether semicarbazide, as such, reaches the tissues, and if it does, to focus research efforts on assessing the genotoxic potential in tissues in which it is found.

Carcinogenicity

Semicarbazide appears to be only a weak carcinogen. It did not cause cancer in rats (Weisburger et al.,1981). In mice, increases in tumours that are commonly observed in untreated mice (tumours of the lung and blood vessels) were seen in treated females but not in treated males. The dose at which these tumours were observed was approximately 100mg/kg bodyweight/day given over a lifetime. It is also noted that semicarbazide is one of the weakest carcinogens among several hydrazines that have been tested in mice (IARC, 1974; Parodi et al., 1981; Cheeseman, 1999). Based on this limited experimental evidence, and taking into account the lack of mechanistic information, it is not possible to conclude whether semicarbazide may pose a carcinogenic risk to humans. In view of the weak carcinogenicity in animals and the very low amounts of semicarbazide in foods, if there is any risk it would appear to be very small.

Conclusions and recommendations

- It is clear that considerable uncertainties about semicarbazide still remain, not only about the extent of human exposure via foods that have been in contact with foamed gaskets but also concerning possible effects *in vivo*.
- Taking into account the information available to date, on the levels in food, intake and toxicology, the risks to consumers eating products containing semicarbazide is likely to be very small.
- The situation for baby foods requires separate comment. The highest concentrations of semicarbazide have been reported to be in baby foods packaged in glass jars and bottles. Due to their food consumption patterns and small body weight, the highest intakes of semicarbazide are likely to be in infants of 6-12

months consuming such foods regularly. Given the present uncertainties in the science, the presence of semicarbazide in baby foods is undesirable.

- The Panel is aware that industry is working intensely on technological means to achieve reduction and/or elimination of semicarbazide. The Panel urges, in the light of the continuing uncertainties in the toxicological data, which do not allow a definitive risk assessment to be made, that industry progresses with maximum speed in these efforts to achieve the goal of reduction of semicarbazide in baby foods and ultimately in other foods.
- Concerning baby foods the Panel also noted that glass jars and bottles with secure seals offer very good protection against microbial risks and this should be taken into account in any risk management decisions that may be made.

References

Industry study (2003). Results Ames-Test Semicarbazide hydrochloride. Report submitted to EFSA by Industry, 29 September 2003.

Cheeseman MA, Machuga EJ & Bailey AB (1999). A tiered approach to threshold of Regulation. Food and Chemical Toxicology 37, 387-412.

CIAA status on semicarbazide Sept. 2003. Document EFSA/AFC/FCM/12-semicarbazide. Letter of 29th September 2003 from D. Taeymans (CIAA) to P. Brunko (DG Health & Consumer Protection).

Commission of the European Communities (1993). Nutrient and energy intakes for the European Community, reports of the Scientific Committee on Food, thirty first series, Office for Official Publications of the EC, Luxembourg.

IARC (1974). IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man: Some Aromatic Amines, Hydrazine and Related Substances, N-Nitroso Compounds and Miscellaneous Alkylating Agents. Volume 4. International Agency for Research on Cancer, Lyon.

Parodi S, Flora SD, Cavanna M, Pino A, Robbiano L, Bennicelli C & Brambilla G (1981). DNA-damaging activity in vivo and bacterial mutagenicity of sixteen hydrazine derivatives as related quantitatively to their carcinogenicity. Cancer Research 41, 1469-1481.

TNO (2003). Oral presentation of results of genotoxicity studies commissioned by EFSA and conducted at TNO, Zeist, The Netherlands, presented at the Scientific Panel meeting on 30 September 2003.

Turrini, A., Saba, A., Perrone, D., Cialda, E. and D'Amicis, A. (2001). Food consumption patterns in Italy. The INN-CA study 1994-1996. *European Journal of Clinical Nutrition*, 55, 1-16.

Weisburger EK, Ulland BM, Nam J, Gart JJ & Weisburger JH (1981). Carcinogenicity tests of certain environmental and industrial chemicals. Journal of the National Cancer Institute 67, 75-88.