INTRODUCTION

The assessment of the risk posed by the exposure to low doses of genotoxic carcinogens is a challenging task in the field of toxicology. Analytical chemistry provides evidence for the presence of minute amounts of established carcinogens in a variety of environmental matrix and common food items, and urge the evaluation of their impact on health. However, low dose effects of chemical carcinogens cannot be directly investigated in experimental systems, because the latter lack the required statistical potency. Animal bioassays infact only allow to detect carcinogenic effects associated with relatively high increases of tumours above spontaneous background, typically a few percent in studies performed following the standard...
protocol with groups of fifty animals per dose. Therefore, in the absence of epidemiological data, the risk to humans from low dose exposures is extrapolated from studies at high doses in experimental animals. This practice requires the extrapolation from animals to humans, and from high to low doses, frequently spanned many orders of magnitude. To this aim several mathematical models have been developed in the second half of the previous century. These models share as default hypothesis the lack of a true, biological threshold in the process of carcinogenesis. This hypothesis, derived from the “one-hit” model in radiobiology, postulates that even a single molecule of a carcinogen has a small, but definite probability to trigger the neoplastic process. The default hypothesis of the absence of threshold for genotoxic carcinogens may be considered conservative, as it does not take into account protective mechanisms which might mitigate the effects of low dose exposure; on the other hand this conservatism compensates for the uncertainties in the carcinogenic process. Therefore, in consideration of the in-built degree of safety offered by the approach, mathematical models have been extensively used in the past years as pragmatic tools for the estimation of the carcinogenic risk for humans.

**APPROACHES FOR CANCER RISK ASSESSMENT**

**Mathematical models**

Dose-response modelling for cancer risk assessment has been performed using a variety of mathematical models, e.g. stochastic, empirical, temporal, based on different statistical and biological premises [1]. Actually, only two of these models have received wide application for the estimation of human risks: the linearized multistage model (LMS), and linear extrapolation. The LMS derives from the model originally proposed by Armitage and Doll for epidemiological data: it assumes that cancer is the result of a multistage process involving multiple independent events, each occurring with a probability which is proportional to the dose of the carcinogen. At very low doses, the dose-response relationship estimated with the LMS model approximates a linear relation at low doses, the dose-response relationship estimated with the LMS model approximates a linear relation with a slope proportional to the dose of the carcinogen. 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useful indication for the setting of tolerable contamination levels could be given by the committee [8], even though tolerable levels are pragmatically unavoidable due to the widespread diffusion of such contaminants. Actually the debate on an alternative approach for cancer risk assessment had already begun within the SCF before it ceased its work at the end of 2002, to be replaced in 2003 by the European Food Safety Authority (EFSA). Therefore at the beginning of its mandate, the Scientific Committee of EFSA has identified within its priority tasks the development of a harmonized, transparent and justifiable approach for the assessment of risks from substances that are both genotoxic and carcinogenic. To this aim an ad hoc working group1 was established early in 2004, which developed a draft proposal adopted by the Scientific Committee of EFSA after a public consultation at the end of 2005 [11].

The harmonized approach of European Food Safety Authority

The approach proposed concerns carcinogenic substances which are genotoxic because of their capacity to interact with DNA, alone or after metabolic conversion. The approach is not intended for genotoxic substances with a thresholded mechanism of action, such as spindle poisons and topoisomerase inhibitors, or acting through indirect mechanisms (e.g. oxidative stress). On the other hand, the approach also covers substances with an unknown mechanism of action, for which a genotoxic mechanism of action is assumed by default. It is defined harmonized, as intended to be used by all the EFSA Scientific Panels and Scientific Committee, possibly providing a unique approach for all the advising bodies within the EU.

Three basic options were considered by EFSA for the development of its harmonized approach: mathematical modelling, ALARA, and the definition of the margin of exposure (MOE). Having the first two options been evaluated as inappropriate by the SCF, EFSA has identified in the MOE a practicable approach for its advising activity on cancer risk. The MOE is defined as the ratio between an established point of the experimental dose-effect relationship (defined as point of departure, POD), and the level of human exposure. The MOE recalls the Margin of Safety (MOS), used in toxicology to derive tolerable exposure levels, which is the ratio between the experimental no observed adverse effect level (NOAEL) and human exposure. However, different from the MOS, in which the NOAEL is used as a surrogate of a threshold, no intrinsically “safe” intake levels can be defined from the MOE, which uses as POD an effective dose level. Even though in principle the MOE does not allow to define safe exposure levels, it is a transparent and effective tool to advice risk managers on cancer risk. The calculation of MOE does not require any mathematical extrapolation, and it takes into account both carcinogenic potency and human exposure: the output is a unique figure, which facilitates the comparison of risks posed by different agents and helps risk managers to set priorities.

In order to derive a MOE for the human exposure of interest, it is necessary to select a point of comparison from the experimental dose-effect relationship. To this aim EFSA has evaluated the suitability of three descriptors of carcinogenic potency: the TD50, the T25, and the Benchmark Dose. The TD50 has been the first synthetic descriptor of carcinogenic potency, used by Gold et al. [12] to establish the large Carcinogenic Potency Database (http://potency.berkeley.edu). The T25 is defined as the chronic dose of carcinogen which halves the probability for an animal to remain tumourless through the standard lifespan. Even though conceptually simple, the calculation of the TD50 is complicated by intercurrent mortality, and it requires the use of a computer programme. A simplified alternative to the TD50 is the T25, defined as the chronic dose associated with 25% of tumours at a specific site, corrected for spontaneous incidence, during the standard lifetime. Differently from the TD50, the T25 is simply derived from the lowest dose producing a statistical significant response, assuming proportionality in the dose-effect relationship [13]. As mentioned above, the T25 is currently adopted in the European Union for setting specific concentration limits for carcinogens in relation to the labelling of preparations. Last, the Benchmark Dose (BMD) is a dose associated with a small but measurable response, typically a 5–10% incidence above control, defined as Benchmark Response (BMR). The Benchmark Dose was originally proposed as a more quantitative alternative to the NOAEL/LOAEL used in general toxicology [14]. The calculation of the BMD is based on a mathematical model fitting all experimental data; to this aim a dedicated software is freely available at the EPA website (www.epa.gov/ncea/bmds.htm). The BMD takes into account the shape of the dose-effect relationship, and thus it results less sensitive compared to other points of comparison to experimental design differences (e.g. selection and span of doses). EFSA has indicated in the BMDL10, i.e. the lower 95% confidence interval of the dose giving a 10% incidence of tumours, the preferred point of comparison for the calculation of the MOE. The BMDL10 in fact requires no or little extrapolation outside the range of experimental data. In case data are insufficient for the calculation of the BMDL10, EFSA has recommended the T25 as an alternative point of comparison, because simple and already in use in the European Union.

Another key step for the definition of the MOE is the selection of an adequate descriptor of human exposure. Uncertainties in the duration and intensity of exposure severely flaw any risk assessment effort; this aspect is particularly critical in relation to the evaluation of risks posed by food borne carcinogens. Indeed the definition of better criteria for exposure assessment and the establishment of an European food consumption database have been early identified as priority tasks by the EFSA Scientific Committee, and are currently tackled by an

1 The author was member of the working group, chaired by Dr. A. Knaap, RIVM, NL.
ad hoc working group. For the time being, EFSA has recommended to follow a flexible approach, i.e. to use whole population estimates for food items widely consumed, and “consumers only” estimates for food items only consumed by a small fraction of the population. In any case it is the chronic, or repeated exposure which has greater relevance for the definition of the MOE.

Whilst the mathematical calculation of the MOE is easy, once that the appropriate point of comparison and exposure of interest are defined, its interpretation may not be straightforward. The comparison of MOE for different substances may allow a comparative evaluation of risks, and their ranking for risk management. However, the size of the residual risk associated with a particular MOE cannot be defined on scientific basis. In particular, it is not possible to use the MOE to define acceptable risk levels. Actually, deciding the acceptability of risk pertains to risk management rather than to risk assessment, because it also takes into account ethic, social, economic aspects. Moreover, as stated above, it is theoretically impossible to identify a MOE associated with no risk at all, as its point of comparison is an effective dose. However, EFSA has concluded that it could be possible to define, on the basis of the MOE, an exposure level which would be of low concern from a public health point of view, and which could be considered as a low priority for risk management actions. Based on current scientific knowledge, EFSA has concluded that a practical threshold is also plausible for genotoxic carcinogens. This does not imply thresholded mechanisms, which are still undemonstrated for genotoxic carcinogens, but the existence of dose levels associated with a vanishingly small incremental risk above spontaneous background. Considering the existence of factors (e.g. detoxification, DNA repair, etc.) which may lead to a substantial deviation from linearity at low doses, EFSA has concluded that linear extrapolation was inappropriate to identify such low risk levels. Rather, uncertainties factors to scale down the risk associated to the point of comparison (BMDL10) have been considered. In particular, EFSA has taken into account uncertainties related to interspecies and interindividual differences in susceptibility, to with the default 100-fold factor used in toxicology has been attributed. Additional uncertainties related to other sources of interindividual variation in susceptibility to carcinogenic effects (i.e. genetic polymorphisms for DNA repair, cell cycle control and other key steps in carcinogenesis), and to the shape of the dose-effect relationship below the BMDL10, have been attributed a further 100-fold factor. Considering both factors, EFSA has concluded that a MOE of 10 000 or greater, when derived from animal data, may be associated to low concern for health and low priority for risk management. Such factor may be increased to take into account weakness in the experimental database. Even though EFSA did not associate a numerical risk estimate to such MOE, it can be observed that the application of a 10 000-fold factor to the BMDL10 would lead to an individual risk of 1x10-5, when estimated with the conservative EPA linear extrapolation method [5].

Prior to its approval, the draft opinion has been published on the internet for a public consultation by stockholders and other interested parties. The most substantial remark, raised by several external observers, concerned the proposed application of uncertainties factors to the benchmark dose to derive an exposure level of low concern. Even though there was a general agreement on the pragmatic use of a 10 000-fold factor to define a minimal risk level, its partitioning in specific uncertainty factors was considered by some scientifically unsound, not adequately supported by current scientific knowledge. Moreover, it was pointed out that the point of departure (BMDL10) of the MOE is an effective dose, not a threshold surrogate, and that the application of uncertainty factors to it does not reduce risk, but merely translates risk to the sensitive part of the population.

Other comments raised on the occasion of the public consultation concerned the possibility of additivity and synergism of effects in cases of mixed exposure, children as a potential sensitive subpopulation, and risk management issues. In this respect it can be observed that current evidence supports the additivity of genotoxic effects in case of multiple exposures, while synergistic effects are rarely observed. This may imply additivity of carcinogenic effects too, and supports the need to keep as low as possible the exposure level to genotoxic carcinogens, independently on the size of MOE. As far as children are concerned, epidemiological data and mechanistic considerations support the hypothesis that children may be especially sensitive to genotoxic effects [15]. Therefore, to account for an increased susceptibility in the young age, adjustment factors for early life exposure to genotoxic carcinogens have been incorporated in the most recent EPA guidelines on cancer risk assessment [5]. Actually the EFSA approach does not mention specifically children as a sensitive group, but it considers an intraspecies uncertainty factor which is of the same size (10-fold) as the adjustment factor recommended for risk assessment of youngest children [15]. Thus it can be assumed that the possible greater sensitivity of children is already accounted for in the 10 000-fold factor applied to the MOE to define minimal risk levels.

CONCLUSIONS

Overall, no basic concern on the pragmatic use of the approach proposed by EFSA was expressed in the open consultation. Admittedly some aspects, in particular the apportionment of the 10 000-fold factor, is still only partially supported by experimental data. This point could be reconsidered in the future on the light of better scientific knowledge on the carcinogenic process. However, despite some limitations, the EFSA approach provides an effective tool to advise risk managers on carcinogenic risk; priorities can be set in a transparent way, and low risk levels identified avoiding the use of formally accurate but scientifically uncertain numerical extrapolations from high to low doses.

Finally, it is important to remind that the approach proposed by EFSA is for substances naturally occur-
ring in food, or present as environmental contaminants of resulting from food processing. The approach is not intended to offer a tool for the registration of genotoxic carcinogens, or otherwise for the definition of acceptable exposure levels for carcinogens deliberately added to food. Even though their banning is a matter of risk management rather than risk assessment, EFSA noted that exposure levels completely devoid of risk cannot be identified with certainty at present: therefore, also in consideration of the possibility of additive effects exerted by genotoxic carcinogens, their presence in food is always regarded as undesirable. Therefore the possibility to define a MOE for genotoxic carcinogens does not preclude the application of risk management measures as recommended by the ALARA, aimed to keep human exposure to genotoxic carcinogens at the lowest level possible.

Submitted on invitation. Accepted on 10 April 2006.

References