Towards a harmonized approach for risk assessment of genotoxic carcinogens in the European Union

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Summary. The EU Scientific Committees have considered in the past the use of matematical models for human cancer risk estimation not adequately supported by the available scientific knowledge. Therefore, the advice given to risk managers was to reduce the exposure as far as possible, following the as low as reasonably achievable (ALARA) principle. However, ALARA does not allow to set priorities for risk management, as it does not take into consideration carcinogenic potency and level of human exposure. For this reason the European Food Safety Authority (EFSA) has identified as a priority task the development of a transparent, scientically justifiable and harmonized approach for risk assessment of genotoxic carcinogens. This approach, proposed at the end of 2005, is based on the definition of the (MOE), *i.e.* the relationship between a given point of the dose reponse curve in the animal and human exposure. As point of comparison EFSA recommends the BMDL10, *i.e.* the lower limit of the confidence interval of the Benchmark Dose associated with an incidence of 10% of induced tumors. Based on current scientific knowkedge, EFSA concluded that a MOE of 10000 or greater is associated with a low risk and low priority for risk management actions. The approach proposed does not replace the ALARA. It should find application on food contaminants, process by-product, and other substances unintentionally present in food. On the other hand, it is not intended to provide a tool for the definition of tolerable intake levels for genotoxic carcinogens deliberately added to food.

Key words: cancer, genotoxic carcinogens, risk assessment, margin of exposure, food safety.

Riassunto *(Verso un approccio armonizzato per la valutazione dei cancerogeni genotossici nell'Unione Europea)*. In passato i Comitati Scientifici dell'UE sono stati restii ad utilizzare modelli matematici per formulare stime quantitative del rischio cancerogeno, ritenendo inadeguate le conoscenze scientifiche per il loro impiego. È stata quindi espressa la raccomandazione di attenersi al principio dell' a*s low as reasonably achievable* (ALARA). Quest'ultimo non permette però di stabilire le necessarie priorità per la gestione del rischio cancerogeno, accomunando cancerogeni deboli e potenti, con ampia o minima esposizione umana. All'inizio della sua attività l'Autorità Europea per la Sicurezza Alimentare (European Food Safety Authority, EFSA) si è quindi proposta di sviluppare un approccio trasparente e scientificamente valido per la valutazione del rischio cancerogeno, da utilizzarsi nell'ambito dell'EFSA ed eventualmente in altri organi consultivi comunitari. L'approccio proposto consiste nella definizione del margine di esposizione (*margin of exposure*, MOE), ossia del rapporto tra una appropriata dose di riferimento della curva dose-risposta nell'animale e l'esposizione umana. Come dose di riferimento l'EFSA ha raccomandato il limite inferiore dell'intervallo di confidenza della benchmark dose associata con una incidenza di tumori indotti del 10% (BMDL10). Sulla base delle informazioni disponibili, l'EFSA ha concluso che un MOE uguale o superiore a 10 000 identifica un basso rischio individuale, a cui attribuire bassa priorità nella gestione del rischio. L'approccio proposto dall'EFSA non sostituisce ma affianca l'ALARA; esso trova applicazione su contaminanti o altre sostanze non aggiunte intenzionalmente agli alimenti, mentre non vuole rappresentare uno strumento per definire concentrazioni tollerabili di cancerogeni genotossici introdotti volontariamente negli alimenti.

Parole chiave: cancro, cancerogeni genotossici, valutazione del rischio, margine di esposizione, sanità alimentare.

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

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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 conservatorism 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 relationship, *i.e.* the model superimposes to linear extrapolation. The LMS assumes that the carcinogen acts with the same mechanism responsible for spontaneous tumors: therefore, as consequence of the additivity of effects, no threshold in the dose-effect relationship is expected. The LMS model has been adopted in the '80s by the US Environmental Protection Agency (EPA) as default model for the estimation of the slope factor, or unit risk, which describes the individual cancer risk associated with lifetime exposure to a unitary dose of carcinogen [2]. Indeed, many of the slope factors of environmental carcinogens reported in the US EPA Integrated Risk Information System (IRIS) database (www.epa.gov/iris) were derived with the LMS. The same model has been adoped by other agencies, including the World Health Organization for the setting of guidelines for drinking water quality [3]. In recent years, however, the LMS has been severely criticized [4], and substituted at the US EPA with a simpler and more transparent approach, based on linear extrapolation from a given point of the experimental dose-effect relationship [5]. The latter method, *i.e.* the linear extrapolation, is also used by several national authorities, *e.g.* in Nordic countries, and has been adopted in the EU as default method for the definition of carcinogenic potency in relation to the classification of dangerous preparations [6].

The as low as reasonably achievable principle

Despite its wide use in the USA, mathematical modelling has never been applied for cancer risk assessment by the Scientific Committees advising the European Commission. In particular, the Scientific Committee on Food (SCF) has expressed serious reservations about the use of mathematical models for estimating the risk at low exposure levels from animal data at high doses. The SCF has pointed out that such models rarely reflect the complexity of the neoplastic process, and that the outcome of the process is severely biased by the mathematical model used [7, 8]. In particular, linear extrapolation from high to low doses may not adequately take into account differences in toxicokinetics/toxicodynamics, efficient detoxification or DNA repair at low exposure level, and vice versa the compensatory cell proliferation at high, cytotoxic doses. All these factors, and the "multi-hit" nature of the carcinogenic process, make plausible a deviation from linearity in the low dose range. Moreover, the numerical estimate of risk extrapolated from the same set of experimental data, to which different mathematical models may fit equally well, may vary up to several orders of magnitude [9]. Due to these reservations, the SCF has never expressed numerical estimates of cancer risk for food carcinogens; rather, the advice given by the SCF to risk managers was to reduce human exposure to the substances as far as reasonably achievable, *i.e.* to apply the as low as reasonably achievable (ALARA) principle [7, 8, 10].

The application of the ALARA priciple in risk assessment has some practical advantadges: it is transparent, widely used and adequately protective for public health, especially when the low exposure level reasonably achievable is associated with a negligible risk (*de minimis* principle). Moreover, the ALARA principle is easily implemented as hazard identification data may suffice, *i.e.* it does not require quantitative data on carcinogenic potency. On the other hand, a cancer risk assessment based on the ALARA principle may be of limited use for risk management. In fact, as the ALARA principle does not take into account the effectiveness of a carcinogen, nor the level of occurrence and/or human exposure, it cannot be used by risk managers to compare risks posed by different substances and to set priorities for future actions.

The inadequacy of the ALARA approach in relation to the advising role of the SCF was highlighted in several circumstances. When evaluating the risk of polycyclic aromatic hydrocarbons in food, for example, no

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). Threfore 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 group[1] 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 unappropriate 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, differently from the MOS, in which the NOAEL is used as a surrogate of a threshold, no intrinsecally "safe" intake levels can be defined from the MOE, which uses as POD an effective dose elvel. 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 TD50 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 associate 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 theorically 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 concer 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 existance of dose levels associated with a vanishingly small incremental risk above spontaneous background. Considering the existance 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 unappropriate 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 stackholders and other interested parties. The most substantial remark, raised by several external observers, concerned the proposed application of uncertainties factors to the benckmark 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 case 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, adjustement factors for early life exposure to genotoxic carcinogens have been incorporated in the most recent EPA guidelines on cancer risk assesment [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 advice 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 assesment, 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 undesiderable. 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.

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