FOOD SCIENCE AND TECHNIQUES

Reports of the Scientific Committee for Food

(Thirty-ninth series)

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food science and techniques

Reports of the Scientific Committee for Food

(39th series)

Opinions of the Scientific Committee for Food on:

The scientific basis of the concept of threshold of regulation in relation to food contact materials

Products derived from bovine tissues, especially gelatin, tallow and di-calcium-phosphate in relation to bovine spongiform encephalopathy

The use of ozone for the removal of unstable elements such as iron, manganese and arsenic from natural mineral water

Dimethyldicarbonate (DMDC, Velcorin)

Phthalates in infant formulas

The calculation of vitamin E content in infant formulas and follow-on formulas

Assessment of novel foods
Part I

Recommendations concerning the scientific aspects of information necessary to support applications for the placing on the market of novel foods and novel food ingredients

Report on:

Principles for the development of microbiological criteria for foodstuffs as covered by the hygiene of foodstuffs Directive 93/43/EEC recommendation

Directorate-General for Industry
1997

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OPINION ON THE SCIENTIFIC BASIS OF THE CONCEPT OF THRESHOLD OF REGULATION IN RELATION TO FOOD CONTACT MATERIALS

(expressed on 8 March 1996)

Terms of reference

1 In the context of food contact materials, the advice of the Scientific Committee for Food (SCF) is requested in relation to the following questions :

i. Are the concept and the limit value of the threshold of regulation introduced by the United States Food and Drug Administration for the analysis of petitions relating to approvals for the use of chemicals as components of food contact materials (equivalent to a per diem exposure of 1.5 µg per person) sufficiently justified from the scientific point of view ?

ii. If not, what are the criticisms of this concept and the resulting value and, if appropriate, which other scientific data are necessary for the Committee to endorse the threshold value established by the Food and Drug Administration or any other threshold value suggested by the Committee for the application of the threshold of regulation concept ?

iii. If the Committee concludes that current knowledge of toxicology does not permit the quantification of the risk for man arising from exposure to chemicals at very low doses, could the Committee nevertheless support the view that for exposure at the level of the threshold value established by the Food and Drug Administration, the existing data show that the risk for man could be considered insignificant ?

iv. If the Committee concludes that at the level of exposure indicated by the Food and Drug Administration's threshold value, the risk for man is not insignificant, could the Committee clarify how it presently evaluates the risk for man arising from exposure to chemicals at very low doses ?

The Committee has been requested to give its opinion on these questions by 31 March 1996

Background

2 Risk assessment of chemicals consumed in small amounts poses problems which are common to a number of classes of food chemicals currently under consideration by the SCF

Of particular note in this regard are flavourings, food contaminants and migrants from food Contact materials. To date, the SCF has examined technical and toxicological data on each individual food chemical in its specific context and has formulated advice using a case by case approach. However, the large numbers of chemicals occuring, used or proposed for use in the areas cited above, the lack of available toxicological data on many of them, and the limited scientific resources both for testing and for evaluation, are forcing advisory and regulatory bodies worldwide to prioritise tasks and devise new strategies in the area of appraisal and regulation of low volume/low exposure food chemicals. The situation described above, together with the recent adoption of a "Threshold of Regulation" policy by the United States Food and Drug Administration (FDA) in relation to food contact materials, and ongoing discussions in the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in relation to flavourings, have prompted the Commission services to ask the SCF for its scientific advice on this matter.

3. It would be easy for regulatory bodies to err on the side of caution and continue to ask for safety data on all chemicals irrespective of intakes. Such a position might be viewed as offering a high level of safety for consumers, but this is not necessarily the case; diversion of regulatory resources into scrutinising one particular chemical sector in great detail may mean that less attention can be paid to other sectors which may be more important to public health. In addition, the Commission and Member States are commited to reducing the use of animals in toxicity testing wherever possible. Against this background, the Commission services have asked, as have other agencies with responsibility for formulating risk management policy, whether, from a scientific standpoint, safety data are actually needed in cases where intakes are known to be very low.

4. Legal frameworks for establishing modalities for risk management of the various classes of chemicals traded within the European Union are at different stages of development and may differ in their approach. At the present time, the priorities imposed on the Commission services by the needs of Member States in the interests of international trade are most pressing in the field of food contact materials. For this reason, the SCF has been asked in the first instance to advise on risk assessment in relation to chemicals consumed in small amounts in the context of food contact materials. The Commission services recognise that the SCF, in addressing the specific concerns relevant for migrants from food contact materials, will need to consider the extent to which some issues can only be resolved by establishing more broadly applicable general principles at a later date.

The threshold of regulation concept

5. The FDA has introduced a Threshold of Regulation (TR) policy in its consideration of petitions for the use of chemicals as components of food contact materials.[4] In essence, the concept behind the TR approach is that there is a level of exposure to non-carcinogenic chemicals in the diet below which, even in the absence of toxicity data, there is reasonable assurance that no adverse effects would occur in man. In practice, by following the TR policy, the FDA will no longer require toxicity data for the acceptance of substances for which the petition shows that migration data obtained under worst case conditions give a dietary concentration below 0.5 ppb and for which there is no reason, on the basis of the chemical structure of the substance, to suspect that the substance is a carcinogen. This dietary concentration of 0.5 ppb equates to a maximum intake of 1.5µg/person/day or 0.03µg/kg bw/day for a 50 kg person, assuming that a person eats 1.5 kg of solid food and 1.5kg of liquid food per day. The FDA has estimated, using quantitative risk assessment (QRA) methodology, that should a substance which is in fact a carcinogen be inadvertently accepted, then at the level of exposure established by the TR value, there would be an 85% probability that the lifetime cancer risk to man would not exceed one in a million. This level of risk is considered by the FDA to be tolerable and reflects the widely held pragmatic view that it is impossible to protect against every unforeseen and exceptional risk from low level exposure to chemicals. The FDA's analysis, which now covers over 500 known carcinogens, did reveal several chemicals which could pose a risk greater than one in a million if present in food at 0.5ppb (e.g. aflatoxin B1 and 2,3,7,8-tetrachlorodibenzo p-dioxin),[5] but these were few and illustrate the point alluded to above that it is impossible to make a practical recommendation for a threshold of regulation if absolute guarantees of no harm are required.

Risk assessment versus risk mangement

6. The Committee noted the clear terms of reference given by the Commission and that the SCF's remit in this task was limited to advice in relation to risk assessment of chemicals to which there may be only low exposures. Risk management should not be considered since this is the duty of the Commission and Member States. Risk assessment involves evaluation of the inherent toxicity of chemicals and estimation of the probability of harm to human health under known conditions of exposure. Risk management involves deciding on the acceptability of any predicted level of risk and what actions, if any, need to be taken to reduce those risks. The FDA, in developing and implementing a threshold of regulation policy as a risk management tool, has

undertaken both roles since risk assessment and risk management fall within their jurisdiction

In the chemicals field, a clear distinction between risk assessment and risk management is often difficult to draw, risk assessment of chemicals is a probabilistic not an actuarial exercise, involving many uncertainties, and so considerable elements of value judgement are called into play

For example, existing approaches utilised by the SCF in advising the Commission on acceptable levels of intake for food additives already embody an element of judgement in selection of the size of safety factors that are deemed acceptable to be applied to no-effect levels derived from toxicity studies in animals or humans Similarly, in addressing the questions put to the Committee by the Commission in the terms of reference set out above, the SCF must comment on QRA methods devised for carcinogens, which often use "worst case" assumptions This already implies an element of selection and rejection of particular scenarios. However, a distinction between the processes of risk assessment and risk management should as far as possible be maintained if the essential scientific basis of the risk assessment process is not to be compromised. In observing this distinction, the role of the SCF must necessarily be limited to advising on whether there is sufficient scientific evidence to define a "threshold of no toxicological concern", rather than extending to a discussion of the issues in terms of a threshold for regulation.

The validity of the threshold concept

7 The concept of thresholds for manifestations of toxicity other than genotoxicity and genotoxic carcinogenicity has been accepted and applied by the SCF over many years Indeed, it is the basis for the setting of Acceptable Daily Intakes (ADIs), Tolerable Daily Intakes (TDIs) and Provisional Tolerable Weekly Intakes (PTWIs) In the absence of human data, these are established by the application of a safety factor to the no-observed-adverse-effect level (NOAEL) determined empirically in a range of animal tests. The design of the specified studies is laid down in international guidelines. The NOAEL identified in the core study among these tests is regarded as that level at and below which there are no observable effects of toxicological concern The principle has been applied routinely on a case by case basis, both for non-carcinogens and also for carcinogens where a consideration of the mechanism indicated the absence of a genotoxic mode of action. The key novel aspect of the present request by the Commission for advice is the possibility of establishing a single, general threshold for toxicological concern and of including within this carcinogens with a potentially genotoxic mechanism of action It is necessary by a stepwise approach to address the

concept of such a threshold and, if considered scientifically valid, to address the level of exposure to which the concept might apply.

I. Chemicals other than genotoxic carcinogens

8 The existence of thresholds for toxicity of compounds other than genotoxic carcinogens is widely accepted within the scientific community and is utilised in very practical ways by regulatory bodies. This acceptance has been based on the observed non-stochastic nature of many dose-response relationships, described graphically by the characteristic sigmoid-shaped dose-response curve, in which no overt toxic effects are observed at the lower doses

These empirical observations have formed the basis of one of the most fundamental principles in toxicology that (to paraphrase Paracelsus) it is the dose which makes the poison Nowadays, empirical observations of thresholds are often supported by mechanistic knowledge which confirms a scientific basis for particular thresholds. This may include, for example, toxicokinetic information, in particular, limitations on absorption, the ability to detoxify by metabolism and conjugation and to excrete rapidly certain toxic compounds and their intermediates, such that little or no biochemical or tissue disturbance occurs; information on receptor-ligand interactions at target sites, which may require a critical concentration of toxic molecules to be achieved before a toxic event is triggered; and knowledge of defence and repair mechanisms which enable cells to cope with short-lived biochemical and structural disturbances, such that no overt manifestation of toxicity occurs

9 While the concept of thresholds has been widely accepted in toxicology, the ability of experimental studies to accurately determine thresholds has certainly not gone unquestioned The life-span of animals compared to man, the numbers of animals used in a study, the choice of dose levels and their spacing relative to one another, the level of detail with which potential changes are examined (organ, tissue, cell or molecular level, by gross pathology, histopathology, electronmicroscopy, and physiological, functional or biochemical parameters), and the appropriateness of the statistical tests applied can all influence the ability of a study to determine the true biological no-effect level Because this is well recognised, techniques have been developed which avoid identifying a NOAEL and instead utilise all the dose-response data to define a dose which causes a small but measurable increase in effect,[3] as for example in the US Environmental Protection Agency's benchmark dose (BMD) concept[4] For risk assessment, either an uncertainty factor is applied to the BMD to derive a reference dose (RfD) which is then used in the same way as an ADI or TDI, or a calculation is made of the margin between the BMD and actual/likely human exposures This may be

particularly valuable in risk assessment of unavoidable environmental contaminants, or with drugs where the margin of safety between therapeutic doses and undesirable toxicity may be quite small. A "margin of safety" approach (i.e. comparisons of low effect levels with exposures) may also be useful in cases such as flavourings, where there are few toxicological data. However, in the wider field of food chemical safety, where usually a safety factor of 100 is applied to a NOAEL, a small imprecision in an experimentally observed NOAEL does not critically influence the overall risk assessment.

10. In the context of discussing whether it is possible to define a threshold of no toxicological concern for non-genotoxic endpoints, the Committee noted that several useful publications and databases are in existence (though not all of them yet available for detailed scrutiny) which have compiled critical NOAELs for large numbers of chemicals.[9,10] Most of these databases are drawn from acute, sub-chronic and chronic studies in rodents. A key consideration therefore is whether endpoints which might give rise to important low dose effects, such as neurotoxic, immunotoxic, endocrinologic and developmentally toxic events, are adequately covered by the studies which make up the bulk of these databases.

To ensure that these aspects are fully taken into consideration and that any threshold chosen would provide an adequate safety margin on their NOAELs, it would be desirable to develop an additional database which focuses on data on the above endpoints. However, it is recognised that the construction of an up-to-date database would require considerable resource and, even with that resource, could not be achieved within the timescale set by the Commission for the initial advice of the SCF in relation to food contact materials (March 1996).

11. As a preliminary step to any future work, the SCF Task Force on Threshold of Regulation has carried out statistical analyses[11] of the distributions of TDIs and PTWIs available from SCF reports on 84 monomers and 276 additives used in food contact materials,[12-16] the 180 ADIs available from the reports of the Joint FAO/WHO Meeting on Pesticide Residues,[17] and the 131 ADIs and PTWIs for food additives and general contaminants set or endorsed by the SCF. While these were not expected to yield particularly low values, it would in any event be necessary to verify that any particular threshold value which might emerge from future discussions was not in conflict with any ADI, TDI or PTWI already established by the Committee. For this exercise they were compared with the approximate intake equivalent (0.03 µg/kg bw/day) of the TR value of 0.5ppb in the diet adopted by the FDA. The results are shown in Table 1. The analyses showed that in no case would the application of the FDA TR value have led to a less conservative outcome for risk assessment (i.e. the level of exposure indicated by the TR value was always less than that indicated by the ADI, TDI, or one-

shown in Table 1. The analyses showed that in no case would the application of the FDA TR value have led to a less conservative outcome for risk assessment (i.e. the level of exposure indicated by the TR value was always less than that indicated by the ADI, TDI, or one-seventh of the PTWI.) With a few exceptions, there was always an appreciable margin between the level indicated by the TR value and the ADI/TDI/PTWI. The exceptions were some pesticides. Ninety-six percent of pesticide ADIs were higher than 10-fold above the TR value, but in the cases of aldrin, dieldrin and heptachlor, having ADIs of 0.1 µg/kg bw/day, the margin was only 3.3-fold, while in the cases of endrin, terbufos and propylenethiourea, having ADIs of 0.2µg/kg bw/day, the margin was 6.7-fold. In the case of monomers for food contact materials, all TDIs were higher than 100-fold above the TR value and in the case of additives for food contact materials, all were higher than 10-fold above the TR value. In the case of food additives and general contaminants, all except one were higher than 100-fold above the TR value. Mercury was only 20-fold above the TR value.

Further analyses using tolerance limits estimated with a 95% confidence level for the lower percentiles of the statistical distributions of ADIs/TDIs/PTWIs suggested that if the application of the FDA TR value were to be extended to unknown compounds falling within the classes of chemical represented by these distributions, the risk that it would lead to exposures higher than any ADI or TDI that might be set was small (ranging from less than two in a thousand to less than one in a million).

12. While the Committee would wish to review the results of more recent studies on key non-genotoxic endpoints before proposing a level of no toxicological concern (as explained in paragraph 10 above), it is the Committee's provisional view that, at the level adopted by the FDA as the basis of a threshold of regulation, few, if any, non-genotoxic and non-genotoxic carcinogenic effects are likely to be expressed and the margin of safety for most such effects is likely to be adequate.

Exceptions to this might be fat-soluble substances which bioaccumulate and/or act through specific receptors, such as 2,3,7,8-tetrachloro-dibenzo-p-dioxin and hormonally active substances; such substances might still exhibit toxicity if taken over a period of time at levels below the FDA threshold value. The Committee also notes that it might be worthwhile considering whether a separate threshold could be proposed for food chemicals which are satisfactorily demonstrated to be non-genotoxic, since this may be useful and, in the present state of knowledge, may be easier to define than a single overall threshold of no toxicological concern covering both non-genotoxic and genotoxic effects. While such a threshold may be useful for the application of regulatory policy in relation to migrants from food contact materials, certain other contaminants, and perhaps some flavourings, the Committee

II. Chemicals which may be genotoxic carcinogens

13 The issue of thresholds for genotoxicity and genotoxic carcinogenicity is a central area of debate in regulatory toxicology. Many scientists feel intuitively that there are probably levels of exposure below which no genotoxic effects would be manifest. But, from a formal point of view, it is necessary to consider whether, at this point in time, there is sufficient scientific evidence to conclude that there is a level of exposure to genotoxic substances below which the likelihood of adverse events resulting in either heritable germ cell mutations or cancer from somatic cell mutations could be excluded with reasonable certainty. Unlike non-genotoxic endpoints where we can draw on strong empirical evidence for thresholds, there are as yet only two experimental studies on low dose administration of genotoxic carcinogens to large numbers of animals and neither of these provided clear evidence of a measurable threshold.[5,6]

In addition, the *in vivo* relationship between carcinogen dose and DNA damage has been found linear over a wide dose range, spanning several orders of magnitude, some examples being aflatoxin B1, benzo(a)pyrene, acetylaminofluorene, 2,4-diaminotoluene and cyclopentapyrene.[7-12]

14 A further complication in the issue of thresholds for genotoxic carcinogens is that the experimental observations on which judgements have to be based are subject to statistical uncertainties, not least because they have to be made against a background incidence of tumours which may be unrelated to exposure to the chemical under consideration. From good experiments it is possible to define statistically a level of exposure which fails to cause any significant increase in particular tumour rates above background rates in controls. At the same time, because one can never prove a null hypothesis, it is impossible to say categorically that exposures at or below this level to the chemical under consideration would never give rise to tumours caused by that chemical. With the exception of the studies already cited,[5,6] experimental animal studies, with their relatively limited numbers of animals, are not designed to establish whether or not thresholds exist.

15. As an alternative to the goal of setting a threshold for expression of genotoxic effects, the occurrence of a carcinogenic outcome can be viewed as a probabilistic event. The fact that, for both genotoxic and non-genotoxic carcinogens, there is a strong dose-response relationship at overtly carcinogenic doses is indirect evidence that at very low doses risks for genotoxic carcinogens probably do become vanishingly small. However, it should be stressed that, from a theoretical point of view, such a reduction in probability does not equate to a threshold in the strict sense of the word since it is

16. Similarly, it is possible to express the issue of carcinogenic risks at very low doses in terms of whether they can be considered negligible risks. It is noted that some governments and regulatory bodies have used mathematical QRA methodology, applied to rodent carcinogenesis bioassays, to derive by extrapolation a dose or level of exposure at which it is predicted that there will be less than a 1 in 10^6, 1 in 10^5, or 1 in 10^4 lifetime risk for man of developing cancer. Such a dose is sometimes referred to as a virtually safe dose or acceptable exposure level. Using such estimates it can then be argued, for example, that exposure to a particular chemical at levels normally encountered in the diet is less risky (e.g. in terms of likelihood of death) than some naturally occurring chemicals in the diet or some other daily activities. However, it should be noted that decisions on whether particular exposure levels are "acceptable" or whether risks are "negligible" or "vanishingly small", involve the risk management elements of social and political value judgements, not just scientific considerations.

17. To date, the SCF has advised on genotoxic carcinogens in the diet (such as contaminants and natural toxicants) on a case by case basis using a "weight of evidence" approach.

This entails forming a judgement about likely potency in man based on all the available toxicokinetic, toxicodynamic and toxicological effects information. In this approach, aspects such as the structure of the chemical, the extent of absorption and retention, its comparative metabolism in test species and man, its range of activity in tests for genotoxicity, preceeding biochemical and pathological changes in tumour target organs, time to tumour formation, location and number of different tumour sites, the number of species and sexes affected, etc, are all taken into account. Such an approach yields a relative and qualitative assessment of carcinogenic risk, rather than a precise quantitative estimate. QRA methodology on the other hand, which does yield a quantitative estimate of risk, utilises only the carcinogenicity bioassay results. The SCF has not so far used QRA methodology and should the SCF decide to do so in the present context, it is recognised it would have repercussions in other fields of SCF activity. The Committee is aware that there is no scientific consensus on the validity of the presently available QRA methodology in predicting cancer risks and there are serious criticisms concerning the results which the mathematical models produce [2]: they may bear only a superficial resemblance to the biological processes underlying carcinogenesis as presently understood, predictions of risk for the same chemical may differ by several orders of magnitude depending on the model used, most models are conservative and could result in over-regulation of chemicals actually posing no risk (an outcome of considerable consequence for naturally occuring contaminants and some existing synthetic chemicals), and the quoting of a precise figure may convey to the public and to those involved directly in risk management a spurious sense of accuracy for the risk estimate. Moolenaar [3] has summarised the policy of the US EPA.

for example, which uses QRA methodology, as follows "EPA policy states that it is not currently scientifically possible to accurately predict risk to humans from experimental data, so no attempt is made to do so. Instead, a plausible upper bound to risk is estimated. EPA states the true risk is expected to be between zero and the upper bound." The SCF does however recognise that, insofar as the most frequently used QRA model, the linearised multistage model, is acknowledged to be conservative, to regulate on the basis of any threshold of toxicological concern derived from it may well ensure protection of public health from the majority of carcinogenic risks in the diet.

Conclusions and recommendations

18 The Committee offers the following views on the questions as posed by the Commission in the terms of reference.

19 *Q1. Are the concept and the limit value of the threshold of regulation introduced by the United States Food and Drug Administration for the analysis of petitions relating to approvals for the use of chemicals as components of food contact materials (equivalent to a per diem exposure of 1.5 µg per person) sufficiently justified from the scientific point of view ?*

Q2. If not, what are the criticisms of this concept and the resulting value and, if appropriate, which other scientific data are necessary for the Committee to endorse the threshold value established by the Food and Drug Administration or any other threshold value suggested by the Committee for the application of the threshold of regulation concept ?

The Committee concludes that the concept behind the threshold of regulation policy, that is to say, the proposition that there is a level of exposure to non-carcinogenic chemicals in the diet below which, even in the absence of toxicity data, there is reasonable assurance that no adverse effects would occur in man, is a sound one. However, the Committee considers that, in the context of the Commission's current request for advice on the threshold of regulation concept in relation to food contact materials, before any firm conclusions could be reached on a dietary limit value for a threshold of no toxicological concern for non-genotoxic endpoints, it would be necessary to conduct an up-to-date review of existing data covering important endpoints of concern which may give rise to effects at low doses, such as neurotoxic, immunotoxic, endocrinologic and developmentally toxic events.

To facilitate progress in future work on this subject the Committee therefore **recommends** that a database be developed that is amenable to interrogation and exploration, which focuses on the above endpoints.

20. It may be worth considering, as a second step, whether it is desirable to set two separate threshold values - one for substances known to be non-genotoxic and a second for substances whose genotoxic potential is unknown. If so, then the setting of a threshold of no toxicological concern for non-genotoxic substances would need to be informed by development of the targeted database recommended above, and would be achievable in the short-term. The possibility of setting of a threshold for substances whose genotoxic potential is unknown would undoubtedly require further fundamental studies addressing the issue of thresholds for the expression of genotoxic activity, and may or may not be achievable in the long-term.

21. *Qiii. If the Committee concludes that current knowledge of toxicology does not permit the quantification of the risk for man arising from exposure to chemicals at very low doses, could the Committee nevertheless support the view that for exposure at the level of the threshold value established by the Food and Drug Administration, the existing data show that the risk for man could be considered insignificant ?*

Qiv. If the Committee concludes that at the level of exposure indicated by the Food and Drug Administration's threshold value, the risk for man is not insignificant, could the Committee clarify how it presently evaluates the risk for man arising from exposure to chemicals at very low doses?

Were a pragmatic decision to be taken by the Commission and Member States to utilise the FDA TR value of 0.5ppb in the daily diet in order to manage the present outstanding problems in the area of food contact materials, such a threshold would, in the Committee's provisional view, protect against most types of toxicity other than genotoxic ones. Exceptions might be substances which are toxic following repeated low levels of exposure due to bioaccumulation and/or interaction with specific receptors. The Committee would be able to give a definitive view on this once the work recommended in paragraph 22 was available. However, the remaining risks from exposure to chemicals at very low doses are most likely to be heritable and carcinogenic risks from genotoxic chemicals. Present scientific knowledge does not allow a definitive conclusion as to whether or not a true threshold exists for genotoxic carcinogens. Given this is the case, some bodies have adopted probability approaches to low dose exposures to carcinogens as the only methods available at present which provide quantitative predictions of risks for man. However, in the Committee's view, the mathematical methods currently used for extrapolating from experimental animal

studies are not sufficiently scientifically well-founded to persuade it that they provide a valid basis for estimating the true risk from very low dose exposures

Nevertheless the Committee recognises that the linearised multistage model favoured in the USA and used by the FDA to derive the TR figure of 0 5ppb incorporates a number of conservative assumptions, aimed at defining an upper bound to low-dose risk estimates, and thereby probably provides protection against a large number of genotoxic carcinogens

22. The SCF's previous considerations of genotoxic carcinogens present as contaminants in food have been largely based on a weight of evidence approach and, in the case of unavoidable contaminants, SCF recommendations have urged reduction of the concentration of the contaminants to the lowest levels technologically achievable or to levels based on analytical limits of detection The Committee recognises that this is a pragmatic rather than a risk-based approach and whilst it is defensible, in that reducing levels of genotoxins in food as low as possible should bring health benefits, it would be desirable to develop methods which will enable the Committee to make risk-based assessments in the future, utilising all the biological evidence Such risk-based assessments would be of more assistance to the Commission for framing of any necessary legislation which at present must be based on the constantly moving target of technical capability To this end the SCF **recommends** that it be given the resources to appraise new developments in risk assessment methodology and, should suitable and robust methods emerge, work towards an appropriate framework of guidelines on how such methods might be applied to the various areas of the Committee's activities.

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Table 1 : Summary of comparisons of ADI's, TDI's and PTWIs' with the intake permitted by the US FDA Threshold of Regulation

Fold above TR[2]	Pesticides	Food Contact		Food Additives
		Monomers	Additives	& contaminants
	(180)	(84)	(276)	(131)
>3.3	100%	100%	100%	100%
>10	96%	100%	100%	100%
>100	77%	100%	95%	99%
>1000	36%	95%	68%	98%
>10000	4%	37%	40%	92%
>100000	1%	10%	12%	69%

Notes

1 PTWIs have been divided by 7 to give daily figure

2 TR taken as equivalent to 0.03 µg/kg bw/day (see text)

3 Numbers in parentheses indicate number of substances in each category

OPINION ON PRODUCTS DERIVED FROM BOVINE TISSUES, ESPECIALLY GELATIN, TALLOW AND DI-CALCIUM-PHOSPHATE IN RELATION TO BOVINE SPONGIFORM ENCEPHALOPATHY

(Expressed on 15 April 1996)

Terms of reference

The Committee was asked to advise the Commission on the extent to which current knowledge of the conditions required to deactivate the agent(s) responsible for spongiform encephalopathies in cattle (BSE) allows specification of treatments for processed food products, which will assure the absence of such agents in processed food products derived from bovine tissues, especially gelatin, tallow and di-calcium phosphate.

Background and Discussion

The Committee noted that since its previous discussion on this issue (100th Meeting of the SCF, 8 March 1996), the British Government announced (20 March 1996) new information relating to a possible link between BSE and Creutzfeldt-Jakob-Disease (CJD) in humans. Following this announcement the Commission, on 27 March 1996, decided on its emergency measures to protect against BSE.[1]

The Committee examined the data made available to it which mainly concerned gelatin. The information on gelatin included interim data from an inactivation study applied to different steps of the production processes. No information was provided to the Committee in a form allowing formulation of an opinion on tallow or di-calcium phosphate at the present time.

The Committee also noted recent considerations made by the Scientific Veterinary Committee and the Scientific Committee on Cosmetology on related issues.

The Committee acknowledges the overall approach which considers the source material, the preparation processes and the routes of exposure. At present the Committee has very limited material in hand to allow evaluation of the production processes or the type and extent of exposure to gelatin, tallow and di-calcium-phosphate from food.

The Committee was informed about manufacturing processes for gelatin as described in an interim documentation of the Gelatin Manufacturers of Europe (GME) for submission to the German Federal Institute for Pharmaceutical and Medicinal Products ("Die BSE-Sicherheit von Pharmagelatine aus Rohstoffen vom Rind", status 28 July 1994). Our understanding of the

[1]Commission Decision 96/239/EC of 27 March 1996, OJ L 78, 28.03.1996.

interim information provided by GME is that gelatin for food use produced in Europe is all made by the processes described in that document, that these processes use source materials regarded to be of very low or no infectivity, that they do not use any source materials from the UK and, should by some rare chance a small amount of infective material from elsewhere be included, its infectivity would be reduced by several orders of magnitude due to dilution within the large production batches and by the heat and the chemical reactions involved in the processes. It was noted by the Committee that scrapie was used as a surrogate for the BSE agent.

Conclusion

Based upon current incomplete knowledge regarding BSE and its possible transmission to humans and the uncertainty about the inactivation of the infective agent, the Committee at present is only able to advise that bovine source materials for these products are to be taken only from geographical areas where BSE does not occur in epidemic proportions.

The Committee urges that data required for a scientifically based risk assessment be generated by relevant bodies. Further research is needed especially to develop specific, sensitive and rapid methods for the detection of the causative agent in biological materials.

OPINION ON THE USE OF OZONE FOR THE REMOVAL OF UNSTABLE ELEMENTS SUCH AS IRON, MANGANESE AND ARSENIC FROM NATURAL MINERAL WATER
(Expressed on the 7 June 1996)

Terms of Reference

To examine the safety of use of the ozone enriched air treatment[1] of certain natural mineral waters[2]

Background

Until about 1970, mineral waters classified by the industry as "very sour" and from which unstable elements[3] such as iron and manganese could not be removed by oxygenation and filtration, were oxygenated and stored in open tanks. After several days, insoluble iron oxide hydrates and calcium carbonate precipitated and the supernatant was decanted, bottled and carbon dioxide was added.

This traditional process is disadvantageous as the removal of iron remains incomplete and has virtually no effect on the concentration of manganese or arsenic. The decrease in the concentration of calcium and hydrogen carbonate ions changes the composition of the water as regards the essential constituents which give it its properties and, furthermore, mineral water kept for a long time in open tanks is very often objectionable from the microbiological point of view.

Subsequently, ozone treatment was found to be much more efficient for the treatment of this class of mineral waters whilst maintaining their essential organoleptic characteristics. Excess ozone would disappear without leaving residues and, at that time reaction products were more or less unknown.

Discussion

Problems linked to the use of ozone enriched air in the treatment of natural mineral waters arise from the formation of reaction by-products, especially bromate and bromoform. The Committee concluded in its opinion issued in February 1989 on the use of potassium bromate as a flour treatment agent, that potassium bromate is a genotoxic carcinogen and that its use

[1] As mentioned in Article 1 of the Commission's proposal for a Directive modifying Directive 80/777/EEC on the exploitation and marketing of natural mineral waters (OJ C 313, 21.11.1994).
[2] As defined in directive 80/777/EEC (OJ L 229, 30.8.1980).

for that purpose should be discontinued (26th Series) Since the advice of the Committee is that levels of genotoxic substances should be kept as low as possible, this would impair the use of ozone if there were no means to suppress side reactions Generally, the use of ozone should be limited to those waters which cannot be treated otherwise

1 Calcium carbonate saturation[3] (1) (2) could be a suitable criterion to restrict the oxidation of unstable elements by ozone enriched air to those natural mineral waters from which e.g. iron, manganese and arsenic cannot be sufficiently removed. The pH of the aerated water must at least exceed 7 for complete removal of iron and 7 2 for removal of manganese (3) (4). At lower pH values of calcium carbonate saturation[3] , calcium carbonate, and occasionally calcium magnesium carbonate, will be precipitated This would alter the composition of the water as regards the essential constituents which give it its properties. In order to prevent these disadvantages, natural mineral waters having a pH value of calcium carbonate saturation below 7 2 could be treated by ozone enriched air

2. Oxidation processes using ozone enriched air should be carried out in closed systems to achieve safe operation (5) The ozone reaction time in reaction tanks should be limited with regard to flow rates, thus providing a means to allow effective control and to minimise undesirable side reactions. Furthermore, side reactions would be suppressed if the pH is reduced during the reaction period by adding carbon dioxide under pressure (6) (7) Excess ozone should not remain in the treated mineral water although it will rapidly decay. After separating iron and manganese oxide hydrates, the water should therefore be filtered over granular activated carbon to remove residual ozone

3 The dosage of ozone enriched air depends on the composition of the natural mineral water and primarily on the ozone demand of the bivalent iron and manganese The ozone dosage should be twice the quantity calculated stoichiometrically in order to compensate for the decomposition of the ozone.

4. Apart from a limited ozone reaction period and a limited dosage of ozone, the most effective measure to suppress the formation of undesirable by-products is a reduction of pH by an additional dosage of carbon dioxide under pressure during the reaction (8) Since it is not possible to predict the optimum level to which the pH should be reduced, it is essential to control the efficiency of the procedure by monitoring the formation of undesirable by-products.

[3] As formulated by the natural water industry

Conclusion

The Committee recognises that the ozone treatment of natural mineral waters may lead to the formation of undesirable by-products. It therefore recommends that the following conditions be met to minimise side reactions which may generate hazardous substances:

The treatment with ozone enriched air of natural mineral waters should be restricted to waters recognised as natural mineral waters and having a pH value of calcium carbonate saturation[3] below pH 7 2

The amount of ozone used for the treatment of natural mineral waters may not exceed twice the demand necessary to oxidise the unstable elements, e.g. bivalent iron, bivalent manganese and trivalent arsenic

The ozone treatment of natural mineral waters should not exceed three minutes

In order to optimise the efficiency of the ozone treatment, treatment plants must be of the closed system type.

Residual ozone must be removed by filtration on granulated activated carbon following the removal of iron and manganese.

The pH of the natural mineral water must be decreased during the reaction with ozone by adding excess carbon dioxide to a level where any side reactions could take place only to a negligible extent

The Committee concluded that residual ozone and the concentrations of undesirable by-products should be in the range of technical unavoidability, should be undetectable by the best available analytical methodology and should be below the levels given below

The Committee is informed that, at the present time, the following limits of quantification are achievable but expects that they will be progressively reduced in line with advances in analytical chemistry

Concentration of residual ozone (9) : 50µg/l
Concentration of bromate (10) (11) : 1µg/l
Concentration of bromoform (12) : 1µg/l

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OPINION ON DIMETHYLDICARBONATE (DMDC, VELCORIN) (RESPONSE TO COMMENTS OF THE FRENCH AUTHORITIES)
(Opinion expressed on 7 June 1996)

Terms of reference

To consider a number of questions raised by the French authorities concerning the SCF opinion on dimethyldicarbonate (DMDC)

Background

DMDC was evaluated by the SCF at its 66th meeting (March 1989) and considered acceptable for the cold sterilization of soft drinks and fruit juices at levels of addition up to 250 mg/l. This decision was published in the 26th Series of Reports of the SCF in 1992 (2) In the Common Position (EC) No 17/94 adopted by the Council on 10 March 1994 (1) on food additives other than colours and sweeteners, DMDC (E 242) is proposed as preservative for non-alcoholic aromatised beverages, alcohol free wine, and liquid tea concentrates at levels of addition of 250 mg/l maximum with residues not detectable

The FDA cleared a food additive petition for the use of DMDC to prevent the growth of yeast in wine on 21.10.1988 and a further petition for the inhibition of yeasts in alcoholfree wine and low alcohol wine by the addition of up to 200 mg/l of DMDC, provided the initial yeast counts are reduced to less than 500 viable cells after an initial filtration or pasteurisation.

DMDC was also evaluated by JECFA in 1990 as set out in the 37th Report No ADI was allocated because the compound disappears after hydrolysis in aqueous media and cannot therefore be determined by analysis It was however judged acceptable as cold sterilising agent up to a level of addition of 250 mg/l

It has been marketed in Germany since 1979 as a processing aid not requiring registration as a food additive However, following a request for inclusion as a preservative in the German Food Additive Regulations and a recommendation of the Senate Commission on food additives and ingredients of the German Research Association (DFG) on its acceptability for this purpose up to a level of addition of 250 mg/l, DMDC was included in the German Food Additive legislation in June 1990

In December 1993 the French authorities submitted a Report of the Section on Food and Nutrition of the Conseil Supérieur d'Hygiène Publique de France which questioned the SCF evaluation of acceptability because in their view some unresolved safety questions still remained

Introduction

DMDC, by virtue of its broad spectrum of antimicrobial activity, has the advantage when used as cold sterilant of fruit juice based beverages of leaving no residues because of its virtually complete hydrolysis in aqueous media to methyl alcohol and CO_2. It does however form minute amounts of reaction products such as carbomethoxylation products of naturally occurring amines, amino acids, sugars and fruit acids (lactic acid, citric acid, ascorbic acid). In the presence of ammonia and NH_4^+ ions very small amounts (less than 25 µg/l) of methylcarbamate are formed. Other reaction products identified were dimethylcarbonate and ethylmethylcarbonate from reaction with methyl and ethyl alcohol. No ethylcarbamate has been shown to arise by transesterification.

The quantities of reaction products found after treatment with 250 mg/l of DMDC were

dimethylcarbonate	less than 0.5 mg/l
Total carbomethoxyderivatives	1.7-5 mg/l
methylcarbamate	less than 25 µg/l
methanol	120 mg/l
CO_2	160 mg/l

Non-gaseous soft drinks and fruit juice based beverages are treated with 125-250 mg/l. Gaseous beverages with sugar require 60-190 mg/l. The process is only efficacious if the inital microbial count is less than 300 viable organisms/l hence prior treatment to reduce initial counts to this level is necessary. The treatment causes no change in colour, odour or taste of the beverage.

Comments of the French authorities

The issues raised by the French authorities highlight certain aspects of the DMDC database considered by the SCF which they perceive to be incomplete. Their comments were as follows:

1. Because of the virtually complete disappearance of DMDC in the treated beverage it is not possible to control analytically the level of addition except through determination of the total methyl alcohol present which includes the naturally present material. As the natural background of methyl alcohol varies with the type of fruit juice used an accurate analytical control of the treatment applied is not possible.

2. There is no disagreement with the SCF view that even with a consumption of 2 l beverage/day for a lifetime the intake of reaction products would be very small.

3. Only 2 types of fruit beverages have been tested namely orange juice and redcurrant juice. As other fruit juices may have different compositions regarding amines, amino acids, sugars and fruit acids it is felt that generalisation of the toxicity assessment with respect to the reaction products to all fruit juices is not justified. Furthermore, no carbomethoxy derivatives of sugars and fruit acids have been identified and toxicologically examined. A total of only 11 carbomethoxy derivatives of amino acids have been examined toxicologically excluding histidine and serine, which are also present in fruit juices. The assumption of linearity of the curve relating the formation of derivatives to the concentration of reactants has only been validated for lactic acid. Apparently this curve is not linear for ascorbic acid. The relationship between reaction products formed and different concentrations of DMDC has not been investigated.

4. Methylcarbamate was shown to be a hepatocarcinogen in the F 344 rat in an NTP study, the NOEL was established at 100 mg/kg b.w. However, methylcarbamate is not carcinogenic in the Wistar rat nor in 2 different mouse strains. It is also not genotoxic in several in vitro and in vivo genotoxicity tests with different endpoints. In the view of the FDA the difference between the exposure to methylcarbamate arising from a consumption of about 20 µg/l beverage and the NOEL of 100 mg/kg b.w. in the lifespan study in the rat is of the order of several magnitudes. In addition the calculated upper bound lifetime risk of cancer from ingestion of methylcarbamate is about 1 in 42 million. The FDA therefore concluded that there is a reasonable certainty of no harm arising from consumption of DMDC-treated beverages. The SCF has concurred with this view but the French authorities doubted the validity of the risk calculation and would prefer to see a comparative metabolism study carried out for DMDC in the F344 and the Wistar rat to explain the different carcinogenic response.

5. In the studies carried out with overtreated orange juice and wine (4000 mg/l DMDC) no estimation of total carbomethoxy derivatives was carried out. Hence their negative outcome cannot be related quantitatively to the amounts found at the technologically permitted treatment level making the significance of the negative results doubtful.

6. The SCF did not pronounce in its report on the question of technological need. This is challenged by the French authorities because they claim to have an industrially effective process using aseptic conditioning of beverages. However, it is admitted that this process needs special equipment and is more expensive but it obviates the appearance of any reaction products with possible toxic potential and would therefore be a suitable alternative.

Conclusions of the Committee

The quantities of reaction products identified are very small and though not every theoretically possible carbomethoxy derivative has been investigated, their toxicological innocuity has been demonstrated in feeding studies on overtreated orange juice. Orange juice was selected as a model beverage because it has been shown to be the most representative fruit juice as regards the natural variability in concentration and occurrence of amino acids, sugars and fruit acids found in different fruit juices. Moreover DMDC is not used on pure 100% fruit juice or on 50% nectars but on beverages containing between 6 and 20% fruit juice only. The amounts of possible reaction products likely to be formed in beverages treated with DMDC at a level of 250 mg/l are therefore about 80-fold lower than were presumably present in the 16-fold overtreated fruit juice.

The assumption that the carcinogenic response to methylcarbamate in the F344 rat is an epigenetic phenomenon is supported by the absence of any genotoxic potential. Moreover, methylcarbamate is not carcinogenic in either the Wistar rat or in two different strains of mouse. For this reason the establishment of a NOEL and a safety factor approach is appropriate. Further metabolic investigations would not affect the conclusions based on the safety factor approach. In making a risk assessment, the difference of several orders of magnitude between the NOEL in the long-term rat study and the residues from the proposed treatment level offers a reasonable assurance of safety for the consumer from exposure to the methylcarbamate formed in treated beverages. The existence of an alternative technology not resulting in any treatment residues is a welcome development but does not offer any scientific reasons to challenge the acceptability of DMDC treatment, the safety of which has been reasonably demonstrated.

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OPINION ON PHTHALATES IN INFANT FORMULAE

(Expressed on 7 June 1996)

Terms of reference

On the basis of the information provided by the Member States, to advise the Commission on the public health implications of the presence of phthalates in infant formulae

Background

The Commission was notified by the UK of the results of an investigation into the contamination of infant formulae with various phthalates and of related findings in the fatty food groups of total diet samples collected as part of the routine UK diet surveys (1,2) Germany and Austria also provided results of investigations into the presence of phthalates in certain food products on their respective markets, but they did not include infant formulae.

The current situation concerning TDIs established by the Committee for phthalates is as follows

Phthalate	TDI mg/kg b.w.	Status of TDI
Di (2-ethylhexyl) (DEHP)	0 05	Full
Butylbenzyl (BBP)	0 1	Temporary
Dibutylbenzyl (DBP)	0 05	Temporary
Dicyclohexyl (DCHP)	0 1	Temporary
Diethyl	0 2	Temporary
Others used in Food contact materials	0 05	Temporary Group restriction for migration

Discussion

Phthalates are industrial chemicals produced in very large tonnages world wide and are known to be environmental contaminants with a high solubility in fat Their presence in infant formulae and other fat containing food products is therefore not unexpected The UK data cover infant formulae, fresh cows milk, milk products, meat, poultry, fish and eggs The German data cover baby foods for children already weaned while the Austrian data cover a wide variety of food products with more detailed data on milk and its products No data on phthalates in human breast milk are available to the Committee but their presence in this medium could be expected because of its high fat content and the occurrence in it of other fat soluble environmental contaminants

In the various infant formulae the levels of total and individual phthalates analysed were comparable. The UK figures show that the estimated intakes of individual phthalates were all below the relevant TDI or temporary TDIs set by the Committee (3,4). Given the uncertainties about which unknown phthalates are included in the measurements of total phthalates and the incomplete knowledge concerning the toxicological profile of such mixtures, the Committee considers that it is inappropriate to compare the level of total phthalates as measured with the SCF group restriction for migration from food contact materials equivalent to 0.05 mg/kg b.w./day.

Earlier reproduction and teratology studies on several phthalates have already revealed effects on the testis and on embryonic development, but only at very high doses, and these studies were included when the Committee established its TDIs.

Since then new evidence on the reproductive effects of phthalates has been published (5,6). In the Sharpe *et al.* study (5) on rats, only a single dose level of butylbenzyl phthalate (BBP) was tested, estimated to range from 0.1 to 0.4 mg/kg b.w./day, *via* the mothers' drinking water throughout pregnancy and lactation. It showed minor effects on testis weight and sperm production. These preliminary findings suggest that reproduction could be affected by BBP at low doses from exposure during prenatal and early postnatal life, but require confirmation. In a study by the National Toxicology Program (NTP) dibutyl phthalate (DBP) was administered in the diet to rats during pregnancy and lactation (6). The male offspring continued to receive DBP for 17 weeks after weaning also *via* the diet. The study showed neither testicular lesions nor reduction of testis weight at a dose level of 279 mg/kg b.w. These effects were observed at higher dose levels.

Conclusions

At the present time, the Committee sees no reasons to change its current TDI or its current temporary TDIs.

It notes that the data provided by the UK for individual phthalates was based on only recently developed methodology.

The Committee concludes that the estimated intakes of individual phthalates are all well below their respective TDI or temporary TDI.

From the UK analytical data the highest estimated intake of BBP was 0.009 mg/kg b.w./day, which is well below the temporary TDI of 0.1 mg/kg b.w. It is also ten times below the level where effects were reported in the Sharpe *et al.* study (5). The Committee considers it important that the findings of Sharpe *et al.* are followed up, but in the meantime it is of the opinion that the levels of BBP found in infant formulae are unlikely to pose a risk to health.

For DBP the highest estimated intake was 0.014 mg/kg b.w./day which is below the temporary TDI of 0.05 mg/kg b.w. The recent NTP study (6) on DBP does not provide any cogent reason to change the temporary TDI. It is therefore the opinion of the Committee that the levels of DBP found in infant formulae do not pose any risk to health

As concerns total phthalates, the Committee considers it inappropriate to compare the level of total phthalates as measured with the SCF group restriction for migration from food contact materials equivalent to 0.05 mg/kg b.w./day

For the risk assessment of phthalates, the Committee stresses the need for further data on the occurrence of phthalates in food in general and for improvements in analytical methodology. Moreover, because it is known that phthalates are ubiquitous in the environment, the Committee is of the opinion that consideration should be given to the environmental aspects by the appropriate bodies.

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THE CALCULATION OF VITAMIN E CONTENT IN INFANT FORMULAE AND FOLLOW-ON FORMULAE
(Expressed on 7 June 1996)

Terms of reference

To advise the Commission on the calculation of the vitamin E content of infant formulae and follow-on formulae.

Background

Directive 91/321/EEC[1] on infant formulae and follow-on formulae specifies that these products shall contain a minimum vitamin E content of 0.5 mg TE[2]/g of polyunsaturated fatty acids (PUFAs) expressed as linoleic acid (Annex I,6 and Annex II,6).

The UK seeks guidance from the European Commission on how this recommendation should be interpreted, particularly because of the more extensive use of PUFA in infant formulae and follow-on formulae. The European Commission has in turn asked for guidance from the Scientific Committee for Food on this matter.

Discussion

It is necessary first to consider if the proposed reference E : PUFA ratio, i.e. the ratio of the amount of vitamin E (mg of d-α tochopherol) to the amount of PUFA in grams, of 0.5 is safe and adequate. From an evaluation of the results of human and animal experiments which diets rich in linoleic acid it was proposed that a ratio of 0.6 is necessary to prevent vitamin E depletion (Harris & Embree, 1963). In different conditions, the minimum symptom-free ratio was found between 0.4 and 0.8, these differences include the particular deficiency sypmtom under examination as well as time of depletion and effects of minerals and other nutrients in the experimental diets (Muggli, 1989). Thus, even if a value of 0.6 may safely be assumed, the current 0.5 value could remain the reference base of calculation.

PUFAs increase the requirement for vitamin E. Highly unsaturated molecules are preferentially peroxized. Holman found that as the number of double bonds increase from 1 to 6, the relative maximum rate of auto-oxidation of individual pure fatty acid methyl esters in

[1] Directive 91/321/EEC on infant formulae and follow-on formulae, O.J. L 175/35, 4.7.91.
[2] TE : Tocopherol Equivalent.

vitro at 37°C were in the ratios 0.025 : 1 : 2 : 4 : 6 : 8 (Holman 1954). The results of in vivo studies have given however slightly different results. By feeding a variety of fats which differ in composition with respect to PUFAs to young, tocopherol-deficient rats and recording the time onset of creatinuria, a symptom of muscle cell membrane damage, Witting et Horwitt have estimated that the rates of peroxidation of mono-, di-, tri-, tetra-, penta-, and hexaenoic fatty acids in terms of relative tocopherol requirements was 0.3, 2, 3, 4, 5, and 6, respectively (Horwitt, 1962, Witting & Horwitt, 1964). Thus, the relative vitamin E requirement, as determined in vivo, can be converted to absolute figures (table below).

TABLE

ESTIMATED MINIMUM REQUIREMENTS OF d - α- TOCOPHEROL ACCORDING TO THE DEGREE OF UNSATURATION OF PUFA PRESENT IN INFANT FORMULAE

Fatty acids	Double bounds	Vitamin E[1] requirements (mg/g fatty acid)
Linoleic	2	0.5
α - linolenic	3	0.75
Arachidonic	4	1
Eicosapentaenoic	5	1.25
Docosahexaenoic	6	1.50

[1] d- α - tocopherol

Conclusion

The Committee therefore recommends that the vitamin E content for infant formulae and follow-on formulae shall be calculated on the basis of the recommended vitamin E content, and taking into account the number of unsaturated bonds given by the PUFA content of the formulae, as it is illustrated by the representative fatty acids in the above table. In no case shall the content be less than 0.5 mg TE/100 kcal.

OPINION ON THE ASSESSMENT OF NOVEL FOODS
PART I

Recommendations concerning the scientific aspects of information necessary to support applications for placing on the market of novel foods and novel food ingredients
(expressed on 7 June 1996)

Table of Contents

1. Introduction

Whenever changes are made to the way in which a food is put on the market, produced or processed or non-traditional ingredients are used, the implications for consumer safety and nutritional value will require consideration. Information will be needed on any issue relating to both these aspects. At present, the issue of food safety in relation to novel foods is under consideration worldwide. The World Health Organization (WHO), the Organization for Economic Cooperation and Development (OECD), and other national and international bodies have addressed both general and specific aspects relevant to the wholesomeness of novel foods. A number of reports outline the philosophies and the developments in this field (see references)

As part of the development of the EU Regulation on Novel Foods and Novel Food Ingredients the European Commission has asked the Scientific Committee for Food (SCF) to develop recommendations concerning the scientific aspects of

I. the information necessary to support an application for placing on the market of novel foods and novel food ingredients,
II. the presentation of such information,
III the preparation of the initial assessment reports.

This report covers task I

2. Categories of novel foods and novel food ingredients identified in the EU regulation

According to the Common Position (EU 25/95) of the Council of October 23, 1995 (95/C 320/01)[1] the EU regulation will apply to the placing on the market of foods or food ingredients which have not hitherto been used for human consumption to a significant degree within the EU and which fall under the following categories

(a) Foods and food ingredients containing or consisting of genetically modified organisms within the meaning of Directive 90/220/EEC[2],

[1] OJ C320 of 30.11.1995, p. 1
[2] OJ L.117 of 8.5.1990, p. 15

(b) Foods and food ingredients produced from, but not containing, genetically modified organisms;
(c) Foods and food ingredients with a new or intentionally modified primary molecular structure;
(d) Foods and food ingredients consisting of or isolated from microorganisms, fungi or algae;
(e) Foods and food ingredients consisting of or isolated from plants and food ingredients isolated from animals, except for foods and food ingredients obtained by traditional propagating and breeding practices and which have a history of safe use;
(f) Foods and food ingredients to which has been applied a production process not currently used, where that process gives rise to significant changes in the composition or structure of the foods or food ingredients which affect their nutritional value, metabolism or the level of undesirable substances

The regulation does not apply to: food additives falling within the scope of Council Directive 89/107/EEC[3], flavourings for use in foodstuffs falling within the scope of Council Directive 88/388/EEC[4], or extraction solvents used in the production of foodstuffs falling within the scope of Council Directive 88/344/EEC[5]

3. Key issues for the assessment of novel foods and novel food ingredients (NF)

3.1 General considerations

Foods are usually complex mixtures of macro- and microconstituents which provide energy and nutrients and contribute to the well-being of humans. They have traditionally been regarded as natural, beneficial and necessary products whose safety and nutritional value need not be questioned. Regulatory approaches to food safety have reflected this attitude and have focused on food additives, processing aids and contaminants of natural or industrial origin. Thus, foods have not hitherto been systematically subjected to nutritional or toxicological evaluation, except in rare cases, where acute toxic effects have been reported in humans (e.g. solanine, cyanogenic glycosides) or in those cases,

[3] OJ L40 of 11.2.1989, p. 27
[4] OJ L184 of 15.7.1988, p. 61
[5] OJ L157 of 24.6.1988, p. 28

where animal studies or human experiences have suggested adverse effects from raw food materials (e g. raw soya flour). This is not to imply that nutritional evaluation of individual foods and of whole diets has not been performed, but that such nutritional evaluations have not been used as a basis for a safety assessment of individual foods. On the other hand, food additives are not permitted in food unless they have been subjected to exhaustive toxicological evaluation

Various foods are known to contain toxic compounds, including mutagens and carcinogens. Some chronic diseases in humans have a dietary element in their etiology. Although it is agreed that some adverse effects of the diet on health are related to the pattern of nutrient intake, the exact mechanisms involved are not known. It is possible that some ill health is due to chronic exposure to constituents of traditional foods. Until recently little attention has been given to this aspect or to the possible role of modifiers of toxic effects (e.g. anticarcinogens) naturally present in foods.

The assessment of the wholesomeness of foods including novel foods and novel food ingredients (NF) presents a number of scientific challenges. Conventional toxicological evaluation methods cannot be applied to foods, because foods present particular difficulties not encountered with the testing of food additives and contaminants *in vivo* and *in vitro*. For example, the amount of food to be incorporated in the diet for animal feeding studies without perturbing its nutritional balance makes the use of conventional safety factors inappropriate for risk assessment and management for any product intended for use as a food or a major food ingredient. Furthermore, traditional metabolic and pharmacokinetic studies are not directly applicable to complex chemical mixtures like foods. The use of mutagenicity and other *in vitro* tests for foods requires special techniques and cautious interpretation of the results

Therefore, alternative approaches for the testing and assessment of the wholesomeness of foods and major food ingredients are needed. The ultimate strategy for combined nutritional- toxicological testing will extend from initial tests *in vitro* and *in vivo* studies in animal models to studies in humans if needed.

3.2 Genetically Modified Organisms (GMO)

Directives 90/219/EEC[6] and 90/220/EEC as amended by directive 94/15/EC set out the information requirements for the safety of the contained use of genetically modified microorganisms (GMM) and the safety of the deliberate release of genetically modified organisms (GMO), respectively. The requirements in these directives are also relevant to GMO covered by the EC Regulation on Novel Foods and Novel Food Ingredients and

[6] OJ L117 of 8.5.1990, p. 1

fulfill basic information requirements needed for the safety assessment of NF. The present recommendations specifically focus on those aspects relevant to human food safety issues.

3.3 Substantial equivalence

The concept of "substantial equivalence" has been introduced by WHO and OECD with particular reference to foods produced by modern biotechnology. In the terminology of the OECD, the concept of substantial equivalence embodies the idea that existing organisms used as foods or as food sources, can serve as a basis for comparison when assessing the safety of human consumption of a food or food component that has been modified or is new. If a new food or food component is found to be substantially equivalent to an existing food or food component, it can be treated in the same manner with respect to safety, keeping in mind that establishment of substantial equivalence is not a safety or nutritional assessment in itself, but an approach to compare a potential new food with its conventional counterpart.

The application of the principle of substantial equivalence can be extended to the evaluation of foods from novel sources and processes. Substantially equivalent NF are thus comparable, in terms of safety, to their conventional counterpart. Substantial equivalence may be established either for the whole food or food component including the introduced "new" change, or it might be established for the food or food component except for the specific "new" change introduced. If a NF has not been found to be substantially equivalent to an existing food or food component, this does not imply that it is unsafe. It just indicates that such a NF should be evaluated on the basis of its unique composition and properties.

The establishment of substantial equivalence is an analytical exercise in the assessment of the relative wholesomeness of a NF compared to an existing food or food component. It contains a dynamic element, as the continuing modification of a food requires that the basis of comparison will evolve in a way that the most recent NF is compared with an appropriate former NF and not necessarily with the most traditional counterpart.

The comparison may be a simple task or be very lengthy depending upon experience with and the nature of the NF under consideration. The technical approach to establishing substantial equivalence will differ between whole animals, plants, microorganims, chemical food ingredients and novel processes and is addressed in more detail under the different classes later in these recommendations.

3.4 Compositional analysis

Analytical studies of the composition of the NF are of crucial importance not only for the establishment of substantial equivalence but also as a prerequisite for nutritional and toxicological assessments. Methods applied have to be standardized and validated to ensure quality and consistency of the data. The analyses and data presented should be based upon sound scientific principles and should be tailored to the nature of the NF. Investigations should focus especially on the determination of the content of critical nutrients (both macro- and micronutrients) and any critical toxicants and anti-nutritional factors which might be either inherently present or process derived.

3.5 Intake

The consumption pattern may show a major change when a NF is included in the diet and thus affects human nutritional status. As it may not be possible to predict such events, a surveillance programme should accompany the marketing of a NF. Such a programme should encompass information on changes in the conditions for processing and preparation as well as effects of possible replacement of other foods or food components of dietary importance. If surveillance reveals changes in those factors which raise concerns regarding wholesomeness, a reappraisal of the acceptability of the NF would be required.

3.6 Nutritional considerations affecting toxicological testing in animals

In the overall evaluation it is of crucial importance to interprete carefully any adverse effects seen in animal studies and to distinguish between toxic effects and those due to nutritional imbalance in the experimental diet. Thus, nutritional and toxicological aspects have to be closely integrated in the assessment of NF. Thorough knowledge of the nutritional properties of the NF (e.g. energy value, protein content, and bioavailability of micronutrients) is needed as a prerequisite of the toxicological testing programme. In designing animal feeding studies, the maximum level of dietary incorporation achievable without causing nutritional imbalance should be the highest dose level, while the lowest dose level should be comparable to its anticipated role in the human diet.

If the predicted usage levels and consumer intakes are likely to be high, the application of the traditionally calculated safety factors employed in safety assessment may create difficulties in designing conventional animal feeding studies with adequate dietary incorporation levels to ensure clearance for use in humans at the anticipated consumption levels. To compensate for the inability of employing reasonably adequate

safety factors any subchronic or chronic animal feeding studies require supplementation by absorption and metabolism studies in animals and eventually in humans

A holistic scientific interpretation of the overall wholesomeness assessment data on a case-by-case basis can provide the acceptable justification for the use of safety factors for NF lower than those traditionally used in safety assessment

3.7 Toxicological requirements

In principle, the toxicological requirements for NF need to be considered on a case-by-case basis. In establishing the need for the provision of toxicological data three scenarios may be considered:

(1) Substantial equivalence can be established to an accepted traditional food or food ingredient, in which case no further testing is needed

(2) Substantial equivalence can be established except for a single or few specific traits of the NF, in which case any further assessment of safety should focus specifically on these traits

(3) Neither partial nor total substantial equivalence can be established, in this case, the wholesomeness of the whole novel food or macronutrient has to be assessed using an appropriate combined nutritional - toxicological approach

If substantial equivalence to a traditional counterpart cannot be established the wholesomeness assessment has to take into account not only knowledge of the identity, chemical structure and physico-chemical properties of the NF but also aspects such as source, composition, potential intake based on the proposed use in the general diet, the potential exposure of particularly vulnerable population groups, and the likely effects of processing. The greater the predicted dietary exposure the more extensive the required toxicological testing programme will have to be.

3.8 Implications of NF to human nutrition

The overall assessment must consider nutritional implications both at expected customary (normal) intakes and at maximum levels of consumption. This evaluation will be guided by a thorough appraisal of relevant literature, compositional analyses, comparisons to consider substantial equivalence, and, if needed, data from investigations in animal models. If a NF is expected to have an important role in the diet then appropriate human nutritional assessment data are needed. Attention should be paid to

the particular physiological characteristics and metabolic requirements of groups such as infants, children, pregnant and lactating women, the elderly, and those with chronic diseases (e.g. diabetes mellitus and malabsorption).

Information will be needed on long term as well as on short term effects of eating the NF. The appropriate information should be derived by combined nutritional and safety post-market surveillance, but additionally consideration should be given to addressing these effects by specific concerns about nutritional quality (e.g. the long term effect of fat replacers on the metabolism of fat soluble vitamins).

3.9 Novel microorganisms used in food

Microorganisms may be used as producers of foods, food ingredients or food additives. Many have a long tradition of safe use in food fermentations. They may be killed in the fermented product or consumed alive with it

By definition, microorganisms with no traditional use in food production in Europe cannot have a substantially equivalent counterpart in Europe and will therefore need to be assessed. Relevant criteria are: containment (e.g. limited to fermentor, remaining alive in food or killed during processing); potential for colonisation of the mammalian gut, potential for toxigenicity as well as pathogenicity in mammals, and whether genetic engineering was applied or not. If genetic modification is employed, the considerations on potential transfer of genetic material from GMM as described in 5.VII become relevant

The safety assessment of a GMM should consider the origin of the newly introduced material, e.g. vectors, regulatory elements, foreign genes including target and marker genes. Two cases have to be considered

- the homologous system (self cloning), where all genetic elements involved are derived from strains within the same taxonomic species,
- the heterologous system, where the donor organism of the genetic elements belongs to a taxonomic species other than that of the recipient

Generally, the segregational and horizontal stability of the constructs are of interest. For self-cloned organisms the concept of substantial equivalence might be applicable in most cases. In heterologous systems both the safety of the gene product in relation to its effects on the food and the effect of the new trait on the properties of the microorganism in the food and, after ingestion, in the gut need to be assessed. The implication of horizontal gene transfer in the gut should be analyzed and evaluated

3.10 Allergenic potential

The potential occurrence of allergic reactions to novel proteins or other constituents of NF should be explored. As a general principle of assessment, the immunological reactivity of individuals who react to the traditional food counterpart should be tested *in vitro* and *in vivo* against the NF. The latter approach may raise ethical issues which must be taken into account. If the novel protein is expressed by genes derived from a source known to be associated with food allergy, sera of people with confirmed allergies to that source can be subjected to specific immunological tests, e.g. Western Blotting or radioallergosorbent test (RAST). If *in vitro* tests are negative, *in vivo* skin prick tests or clinically supervised double blind placebo controlled challenges in these people may be performed. All studies should comply with relevant elements and ethical principles of guidelines on Good Clinical Practice and Good Laboratory Practice.

A number of factors can serve as indicators of the potential allergenicity of novel proteins, such as sequence epitope homology with known allergens, heat stability, sensitivity to pH, digestibility by gastrointestinal proteases, detectable amounts in plasma, and molecular weight. Additional evidence might emerge from pre-marketing human results and reports of workers' sensitizations.

New approaches are needed to assess the potential allergenicity of NF in humans. In the present state of knowledge, the allergenicity of a novel food from a GM source should include consideration of the allergenic potential of the donor and of the recipient organism.

3.11 Assessment of marker genes.

Marker genes are used as "tags" to identify and to select those cells of plants or microorganisms which have been transformed successfully by genetic modification. Normally they are not supposed to play a role of their own in the final product or NF. The marker genes, presently used most frequently in plants, are those conferring resistance to antibiotics or increased tolerance to herbicides. Others confer heavy metal tolerance or phenotypic and biochemical selection. The requirements for evaluating the safety of marker genes are basically similar to those applicable to the safety evaluation of any other foreign genes.

The assessment in plants needs to consider:

- the marker gene itself and the product it encodes;
- the methods for analyzing and quantifying the marker gene and its expression products in the food,
- the potential toxicological and/or nutritional effects related to the function of the marker gene,
- the potential for horizontal gene transfer to gut microorganisms

The use of marker genes in microorganisms, especially those genes conferring antibiotic resistance, has to be assessed in relation to the host organism, the biological containment established by the genetic construct, the possibility of colonization of the human gut by these GMO, and the relationship between the efficacy of antimicrobials and the acquired resistance.

It can be foreseen that a list of approved marker genes can be developed based upon an evaluation of their primary effects on the host organism. Their secondary effects on the host will depend, among other factors on the insertion site in the host DNA and will need an assessment on a case-by-case basis, although there is no reason to suppose that the potential for secondary effects is greater for marker genes than for any other inserted genes.

4. Scientific classification of novel foods for the assessment of whole-someness

Foods and food ingredients which fall within the scope of the EU Regulation on Novel Foods and Food Ingredients are very diverse (see Section 2). To facilitate safety and nutritional evaluation, six classes of NF have been identified. These differ in complexity and in the issues that need to be addressed

For the purpose of these recommendations, the term "plants" covers also seaweed. The term "animals" includes fish and shellfish, and the term "microorganism" encompasses bacteria, fungi (including yeasts), and micro-algae (viruses and plasmids are outside the scope of these guidelines)

Class 1: Pure chemicals or simple mixtures from non-GM sources

This class comprises foods and food components that are single chemically defined substances or mixtures of these which are not obtained from plants, animals or microorganisms that have been genetically modified. Two sub classes can be identified

1.1 the source of the NF has a history of food use in the EU
1.2 the source of the NF has no history of food use in the EU

Class 2: Complex NF from non-GM sources

This class comprises complex NF which are or are derived from sources which have not been genetically modified. Intact plants, animals and microorganisms used as foods as well as food components (e.g. complex carbohydrates, fats, proteins or those substances collectively described as dietary fibre) are included. Two sub classes can be identified:

2.1 the source of the NF has a history of food use in the EU.
2.2 the source of the NF has no history of food use in the EU

Class 3: GM plants and their products

GM plants can be consumed directly as unprocessed foods or after having been processed into foods and food ingredients including pure chemicals. This class of NF includes all such foods and food ingredients. Two sub classes can be identified.

3.1 the host plant used for the genetic modification has a history of use as food or as a source of food in the EU under comparable conditions of preparation and intake.
3.2 the host plant used for the genetic modification has no history of use as food or as a source of food in the EU under comparable conditions of preparation and intake.

Class 4: GM animals and their products

GM animals can be consumed directly as unprocessed foods or after having been processed into foods and food ingredients including pure chemicals. Products directly produced by GM animals (e.g. eggs, milk) can be consumed either processed or unprocessed. This class of NF includes all such foods and food ingredients. Two sub classes can be identified:

4.1 the host animal used for the genetic modification has a history of use as food or as a source of food in the EU under comparable conditions of preparation and intake.
4.2 the host animal used for the genetic modification has no history of use as food or as a source of food in the EU under comparable conditions of preparation and intake.

Class 5: GM microorganisms and their products

Living GM microorganisms may be used in food production or in the production of food ingredients. This class includes all NF which are, or are produced using, GM microorganisms whether or not there are any living cells in the NF as consumed. Two sub classes can be identified:

5.1 the host microorganism used for the genetic modification has a history of use as food or as a source of food in the EU under comparable conditions of preparation and intake

5.2 the host microorganism used for the genetic modification has no history of use as food or as a source of food in the EU under comparable conditions of preparation and intake.

Class 6: Foods produced using a novel process

This class comprises foods and food ingredients which have been subjected to a process not currently used in food production. Novel processes for food production may encompass for example new types of heat processing, non-thermal preservation methods, new processes to chill or freeze products, to dehydrate products, and the application of new processes catalyzed by enzymes. According to the scope of the EU regulation, the resulting product is only considered to be a NF, if the process results in changes in the chemical composition or structure of the food or food ingredient, which affect its nutritional value, metabolism or level of undesirable substances.

The association between the described classes and the categorization in the EU Regulation on Novel Foods and Novel Food Ingredients is outlined in Table 1

5. Identification of essential information for assessment of wholesomeness

In this section structured schemes are provided to identify the types of information that are likely to be required to establish the safety of particular classes of NF. It is recognized that no formalistic approach can cover adequately all NF, and these schemes are therefore provided for guidance only. If other information is available or relevant for the assessment, it should be submitted. However, if it is proposed to omit certain information from a dossier requested in any of the schemes, the scientific justification for this should be given. The results of any investigations relevant to safety assessment which have been carried out must be reported

In the assessment of NF the focus is the novelty *per se*. Chemical or microbiological contaminants of NF not specifically related to the novelty are not addressed in these recommendations. Similarly, the presence of microbial toxins and microbial or viral infective agents is not considered unless this is a consequence of the novelty.

The identification of essential information for assessment is guided by the division into six classes described in chapter 4. After allocating a NF to a class or subclass, the attached Table II can be used to determine which of the structured schemes I-XIII should be consulted to provide the information required to support its safety and nutritional evaluation.

In the following, the information requested in each particular structured scheme is specified in more detail.

I. Specification of the NF

Specification of the origin and the composition of the NF is needed to ensure the identity between the product tested/evaluated and the product to be marketed. In the design of the specification, parameters most relevant to characterize the product from a safety and nutritional point of view should be considered.

Such parameters include species and taxon, as well as chemical composition relating particularly to nutritional properties and possible antinutritional/toxicological concerns. Taxonomic identity should be established according to referenced and internationally accepted principles and deviation from such principles should be explained.

Information on the availability of specified reference material should be submitted.

II. Effect of the production process applied to the NF

In principle, this scheme applies to all NF which have been processed during production. The description of the technical details has to be sufficiently detailed (i) to permit a distinction between novel and existing processes, and (ii) to predict whether the potential of the process to introduce physical, chemical and/or biological changes in the food might have an impact on essential nutritional, toxicological and microbiological parameters of the final product.

The assessment of new technologies needs to address any organic and inorganic residues or contaminants derived from apparatus and equipments or from chemical, physical or biological aids used in the novel process. Critical aspects of the production process in

relation to NF are those, which ensure that the final products of the described process comply with the specifications given under scheme I.

As regards hygienic parameters, these are not included in the assessment of NFs but are covered by Directive 93/43/EEC[7].

The assessment will focus on the food product resulting from the novel process on a case-by-case basis. The ultimate aim of assessment will be the evalution of the process in a wider sense without the need for actually testing and assessing each conceivable food/process combination. This implies a broader strategy in which representatives of relevant food classes, processed by the novel food process, should be compared either to untreated counterparts or to counterparts which have been processed in a related traditional manner.

III. History of the organism used as the source of the NF

The novelty of food plants, food animals or food microorganisms in relation to these guidelines is defined by their novelty in the European food supply. If species/taxons of plants, animals or microorganisms have had no generally recognised use in the diet of any of the EC countries according to national dietary records, the species/taxon is considered new, and a full description is needed to assess its future role in the European food supply. This should include information on the past and present use of the plant, animal or microorganism and its products in the food supply in other parts of the world. Such information should also include

- past and present methods to obtain raw materials and food, e.g. by raising, harvesting, slaughtering, and capture,
- procedures for fermentation and preparation,
- description of transport and storage conditions, and
- its traditional role in the diet at locations outside the EU.

IV. Effect of the genetic modification on the properties of the host organism

The information gathered through this scheme focuses on the effects of the genetic modification on the properties of the GMO compared to the host organism. It differentiates between intended and unintended effects. In the latter case, special attention should be given to any nutritional, toxicological, and microbiological impact on the foods.

[7] OJ L.175 of 19.7.1993, p. 1

GM plants. The principles for evaluating GM plants and their products are similar to those valid for non-GM plants and their products. The safety evaluation of a GM plant may be a simpler task than the evaluation of a novel non GM plant, if the nonmodified organism is a traditional food plant and the alteration has occurred by means of a precisely defined process of genetic modification. In this case, the safety assessment can focus on the results of the genetic modification.

Where the genetic modification results in a new phenotype, the compositional consequences of this modification should be defined and tested. If, for example, a genetically modified plant is so designed as to express a naturally occurring insecticide, encoded by a gene derived from another organism, and has therefore become resistant to certain insect pests, then the toxicological profile of the introduced insecticidal component needs to be determined. The safety of this modification of the chemical composition can be evaluated by standard toxicological procedures, it should include an assessment of the potential allergenicity. In addition, secondary effects (positional effects) have to be taken into consideration. These effects of the insertional event, e. g. the insertional mutation itself or a genomic rearrangement will influence the overall outcome of the genetic modification. A knowledge of the normal toxin production in the plant and the effect on it of various growth and culturing conditions, to which the GM plant is subjected, as well as knowledge whether the new gene product appears in the final food, is essential. The same reasoning applies to nutritionally important components especially in food plants.

Essential steps of the safety evaluation are therefore:

- characterisation of the parent food organism;
- characterisation at the molecular level of the nature of the genetic modification including insertional position, copy number and biochemical expression level;
- establishment, as far as possible, of substantial equivalence between the parent food organism and its new derivative through chemical and phenotypic analysis;
- if substantial equivalence cannot be established, conventional safety studies on specific chemicals occurring in the food due to the phenotypic changes involving either the new product of the new gene or the safety of inherent natural toxins now present in altered amounts. The potential allergenicity of the new components also needs to be addressed.

GM animals. The general principles established for the safety evaluation of GM plants apply also to GM animals. The safety assessment will initially address the establishment of substantial equivalence between the parent organism and the GM organism focussing on primary and secondary effects of the genetic modification process. For example if the modification is directed towards changing the globulins in cow's milk to a more

"human" type, the new globulins have to be assessed. Another example may be a fish genetically modified to produce an antifreeze protein. The safety of this chemical modification can be evaluated by conventional toxicological strategies and should also include an assessment of the allergenicity aspects.

GM microorganisms. In compliance with the provisions set out for GM plants and GM animals, the parent microorganism, which is the subject of genetic modification has *a priori* to be recognized either as a microrganism with a tradition in food fermentation in the EU, as a non-pathogenic, biologically advantageous human intestinal commensal, or as a traditionally used production organism for foods, including food additives and technical aids, to simplify the evaluation procedure. In other cases, not only the genetic modification but also the parent microorganism needs to be assessed as being novel

V. Genetic stability of the GMO used as NF source

The question concerning genetic stability relates to the structural and local maintenance of the introduced genetic material and to the gene expression in the GMO.

VI. Specificity of expression of novel genetic material

This scheme relates to the factors involved in regulation of gene expression, for instance organ/tissue specificity, conditions of repression and activation

VII. Transfer of genetic material from GMO

Based on current knowledge, considerations of gene transfer from GMO in the human gut focus on microorganisms. Horizontal gene transfer among microorganisms is well established and has therefore to be considered in food safety assessments. One aspect of biological containment is the possible transfer of genetic material from GM microorganisms to the human gut microflora. There are different possibilities for addressing this aspect in an experimental setting, e.g. animal or *in vitro* gut models

In assessing the food safety consequences of gene transfer, the nature of the gene and its product, the frequency of the transfer, and the level of expression in transformed gut microorganisms should be taken into account. Transfer of genes from plants to microorganisms is a theoretical possibility, the consequences of such an event should be considered

VIII. Ability of the GMM to survive in and colonise the human gut

The genetic modification might facilitate survival during passage through the intestines and colonization of the human gut. Antagonistic and synergistic effects on the composition of the intestinal flora may occur and have an influence on human health. Therefore, experimental data are required on the respective properties of the GMO.

For living GMM in food attention should particularly focus on their capability to survive in and colonize the gastrointestinal tract and to maintain their genomic stability. For this assessment *in vitro* and *in vivo* gut models mimicking the human situation as closely as possible may be needed. Aspects relating to pathogenicity and gastrointestinal immunity need special consideration.

IX. Anticipated intake/extent of use of the NF

Projections of anticipated intakes are needed to evaluate the dietary and nutritional significance of NF. This assessment will naturally draw upon information on the nature of the NF and its anticipated uses based upon its properties e.g. as a fat replacer.

X. Information from previous human exposure to the NF or its source

Documentation on previous use of the NF source in the EU or the NF source and /or the NF in other parts of the world is important to establish a baseline for assessment. However, history of food use outside the EU is not of itself a guarantee that the NF can be safely consumed in the EC. The information should deal with such aspects, where traditional handling and preparation of the plant, animal or microorganism prevent misuse or adverse short and long term health effects, for example those due to inherent antinutritional/toxic factors. In many cases, necessary precautions are reflected in the corresponding regional and cultural habits.

XI. Nutritional information on the NF

The overall assessment should, as indicated above, include a systematic review of the NF's composition, preparation and role which it is expected to have in the diet. Such an assessment with a review of relevant published material would enable an appraisal of substantial equivalence to a traditional food or food component.

If substantial equivalence cannot be established appropriate preliminary assessments should be made in animal models to establish some aspects of nutritional quality but full nutritional assessment needs to be done in human subjects. Such studies should be based on well defined hypotheses with clear nutritional and metabolic outcomes relevant to the NF, to its dietary context, and to the anticipated consumer group.

Nutritional consequences should be assessed at normal and maximum levels of consumption, and the nutrient compositional data should take into account the effects of storage, further processing and cooking. The effect of antinutritional factors (e.g. inhibiting mineral absorption or bioavailability) on the nutritional value of the whole diet should also be assessed.

The numbers involved in study groups should ensure that the study has adequate statistical power. All studies should comply with relevant elements and ethical principles of guidelines on Good Clinical Practice and Good Laboratory Practice.

In some circumstances it is envisaged that plans should be provided for post-market surveillance for possible long term effects of the NF.

XII. Microbiological information on the NF

In addition to toxicological and nutritional safety wholesomeness of a NF embraces microbiological safety. Generally, the intentionally used source organism for the NF has to be recognised as a non-pathogenic, non-toxigenic microorganism of known genetic stability which does not affect the desirable properties of the normal intestinal flora. The examination of a NF should include a characterization of the microorganisms present and the analysis of their metabolites.

XIII. Toxicological information on the NF

This scheme covers the set of toxicological information needed to assess the NF. The range of scenarios can extend from foods for which substantial equivalence can be

established to foods for which substantial equivalence cannot be established and which, therefore, require an appropriate nutritional-toxicological testing program

If substantial equivalence to a traditional counterpart cannot be established, the safety assessment based on a case-by-case evaluation must consider the following elements

- consideration of the possible toxicity of the analytically identified individual chemical components,
- toxicity studies *in vitro* and *in vivo* including mutagenicity studies, reproduction and teratogenicity studies as well as long term feeding studies, following a tiered approach on a case-by-case basis,
- studies on potential allergenicity

In the case of novel microconstituents and isolated novel food components, which differ by identifiable characteristics from traditional foods or for defined novel products obtained from genetically modified organisms, it is possible to restrict testing to only those products or substances rather than the whole NF. In some cases, the testing of the novel property would have only marginal nutritional implications for laboratory animals so that the traditional toxicological approach can be applied for establishing safety.

Most of the defined chemical substances can probably be tested for their safety similarly to food additives by utilising conventional methods of safety evaluation as described in the SCF Report No. 10. This implies the use of conventional toxicological testing procedures applied in a tiered sequence. This would involve initial mutagenicity studies and an appropriate feeding study in a rodent species with an exhaustive investigation of all relevant toxicological parameters. Furthermore, if warranted by structural or exposure considerations, additional investigations should be undertaken covering all the usual toxicological endpoints including metabolism, toxicokinetics, chronic toxicity/ carcinogenicity, reproductive function, teratogenicity, and possibly neurotoxicity and immunotoxicity.

Novel macroconstituents or NF which are not substantially equivalent to traditional counterparts will require a testing programme depending on the toxicological concerns raised. In general, this programme should include at least a 90 day feeding study in a rodent species, whereby special attention is paid to the choice of doses and the avoidance of problems of nutritional imbalance. These constraints may require a different way of conducting toxicological studies and interpreting their results (see 3.6).

The potential for mutagenicity needs investigation. Any *in vitro* mutagenicity studies will need to cover the usual major endpoints. Special technical problems may be encountered in testing novel macroconstituents in *in vitro* mutagenicity test systems, particularly because of effects of the NF or its constituents on the growth medium, the test cells or

the test organisms, unrelated to mutagenicity. There may be cases where feeding studies in a second species and an investigation of effects on the composition of the intestinal flora are needed. Also chronic toxicity/carcinogenicity studies may be necessary. The allergenic potential needs also to be investigated.

6. Review of recommendations

The area of novel foods is developing rapidly. The science and technology is making enormous advances and many countries and international organisations are elaborating procedures and guidelines for the safety assessment of novel foods. In the light of these developments, and of experiences gained in applying its current recommendations, the SCF will reconsider these recommendations after five years.

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8 Glossary

This glossary is intended to explain the way in which various terms are used by the SCF in its recommendations rather than to provide precise scientific definitions

Copy number:	the number of times a particular coding sequence is present in the genome
DNA:	deoxyribonucleic acid, which is present in all living cells and contains the information for cellular structure, organisation and function
Donor:	organism from which genetic material has been obtained for subsequent transfer
Epitope:	distinct region of an antigen which is recognized by the combining site of an antibody
Expression:	manifestation of a characteristic that is specified by a gene
Food allergy:	adverse immune (IgE)-mediated reactions to foods occurring in susceptible individuals
Gene:	smallest portion of a DNA molecule that contains sufficient heritable information to code for a particular trait or function of an organism

Food allergy:	adverse immune (IgE)-mediated reactions to foods occurring in susceptible individuals
Gene:	smallest portion of a DNA molecule that contains sufficient heritable information to code for a particular trait or function of an organism
Genetic modification:	alteration of genetic material using the techniques defined in Directive 90/220/EEC
Genetically modified organism (GMO):	an organism in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination
Genetic stability:	the degree to which the genetic make-up of an organism is inherited unaltered by subsequent generations
Genome:	the total of the genes of an organism
Host:	organism into which heritable genetic material prepared elsewhere has been introduced
Immunoassay:	method of measuring using antibodies to detect concentrations of unknown substances
Insertion:	addition of one or more nucleotide base pairs into a DNA molecule
Organism:	any biological entity capable of replication or of transferring genetic material
Plasmid:	circular piece of extrachromosomal DNA in bacteria and certain other organisms, capable of replicating independently of the chromosome
Position effect:	unintentional effect caused by the insertion of a gene which interferes with the normal function of another gene
Vector:	self-replicating DNA molecule modified to transfer a foreign DNA segment into the host genome

Table 1 Association between the categorization in the EU Regulation on Novel Foods and Novel Food Ingredients and the SCF recommendations

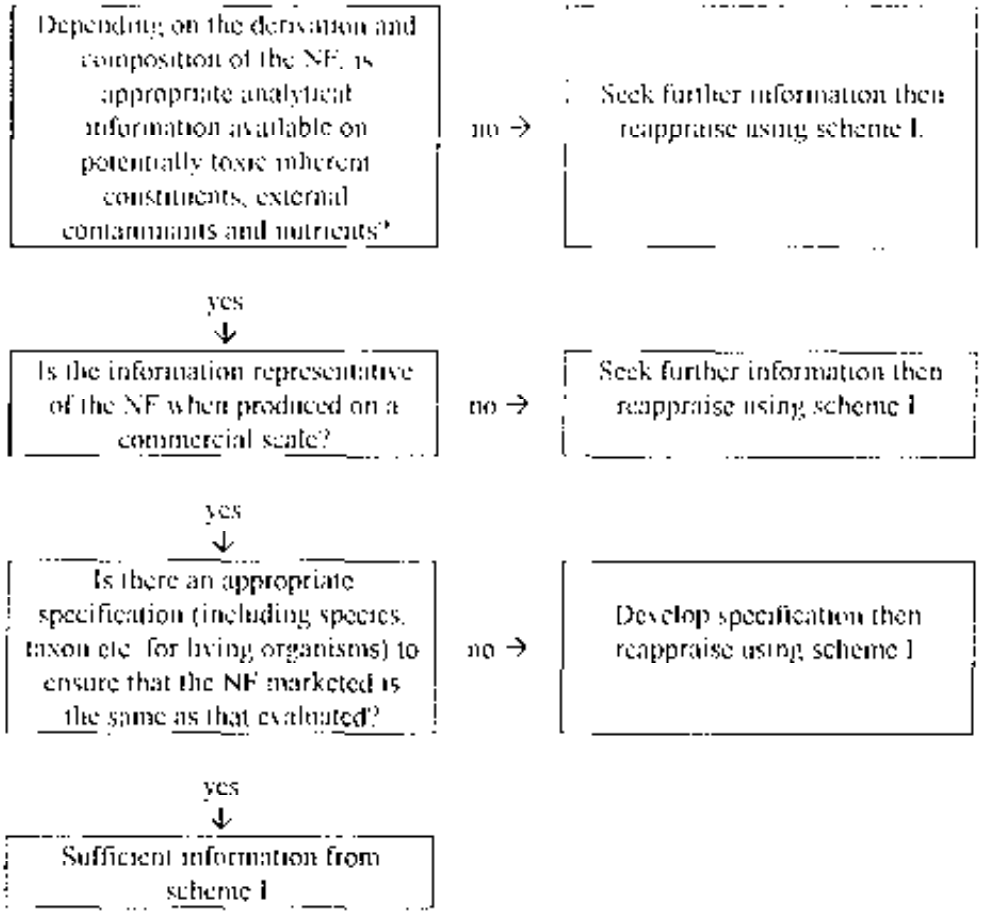
EU Regulation Art. 1 (2)

		a	b	c	d	e	f
Class 1	Pure chemicals or simple mixtures from non-GM sources			X	X	X	
Class 2	Complex NF from non-GM sources				X	X	
Class 3	GM plants and their products	X	X				
Class 4	GM animals and their products	X	X				
Class 5	GM microorganisms and their products	X	X				
Class 6	Foods produced using a novel process						X

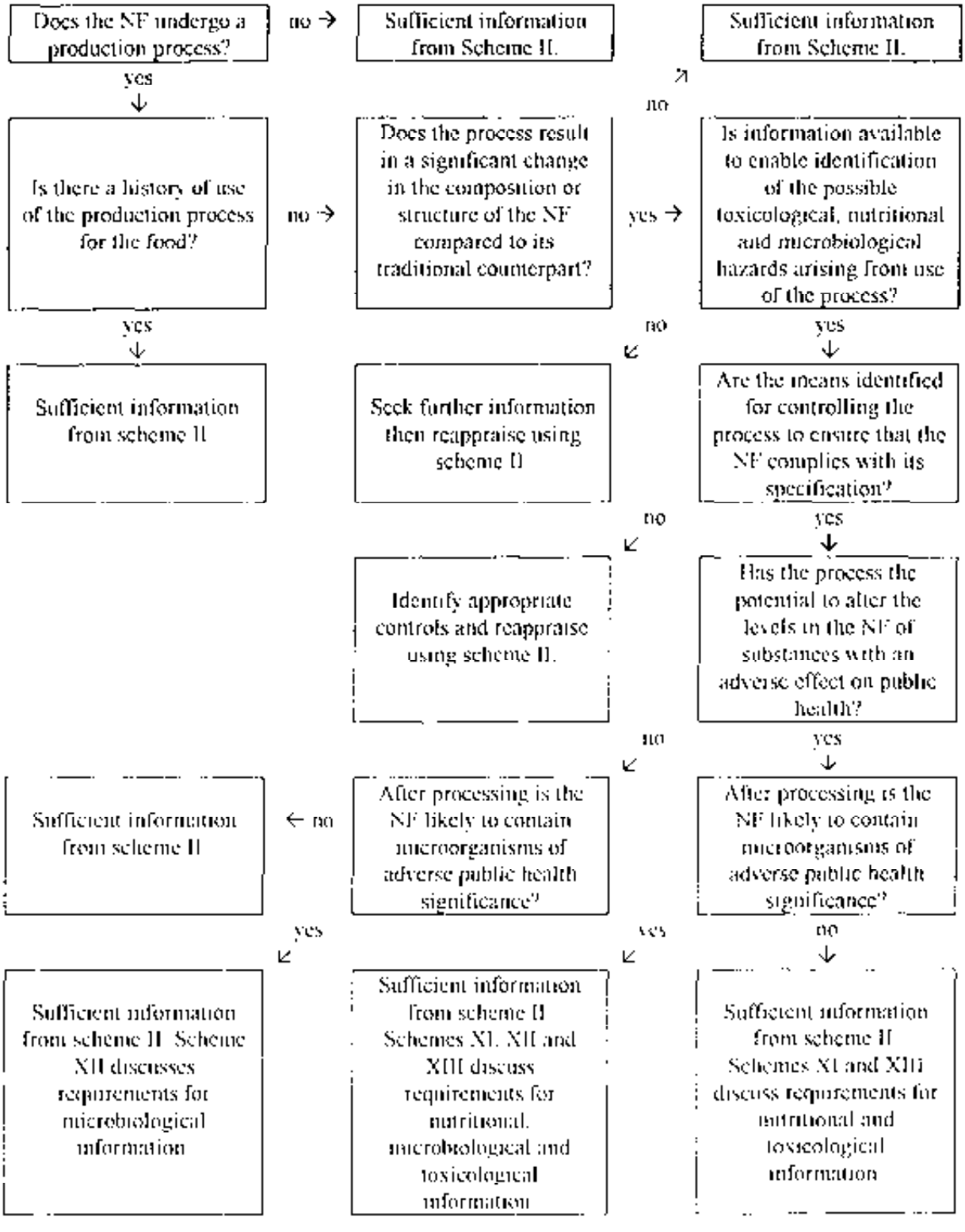
Table II: Index
to structured schemes to be followed for each class of NF

	Class of NF	1.1	1.2	2.1	2.2	3.1	3.2	4.1	4.2	5.1	5.2	6
	Structured scheme											
I	Specification of the NF	X	X	X	X	X	X	X	X	X	X	X
II.	Effect of the production process applied to the NF	X	X	X	X	X	X	X	X	X	X	X
III.	History of the organism used as the source of the NF.	X	X	X	X	X	X	X	X	X	X	X
IV.	Effect of the genetic modification on the properties of the host organism					X	X	X	X	X	X	
V.	Genetic stability of the GMO					X	X	X	X	X	X	
VI	Specificity of expression of novel genetic material					X	X	X	X	X	X	
VII	Transfer of genetic material from GM microorganisms					X	X	X	X	X	X	
VIII	Ability to survive in and colonise the human gut									X	X	
IX	Anticipated intake/extent of use of the NF	X	X	X	X	X	X	X	X	X	X	X
X	Information from previous human exposure to the NF or its source	X		X		X		X		X		X
XI	Nutritional information on the NF	X	X	X	X	X	X	X	X	X	X	X
XII	Microbiological information on the NF	X	X	X	X	X	X	X	X	X	X	X
XIII	Toxicological information on the NF	X	X	X	X	X	X	X	X	X	X	X

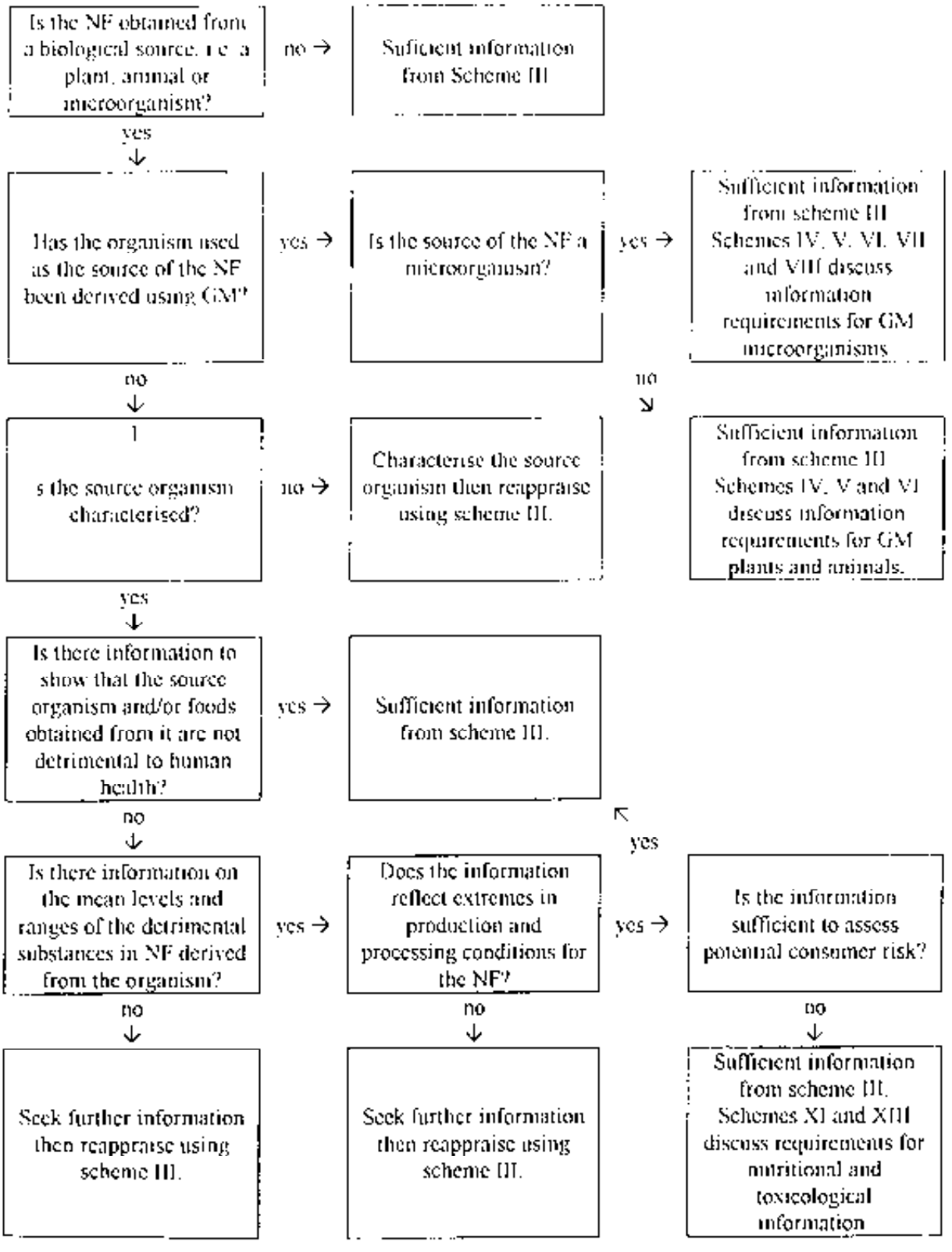
I. Specification of the NF



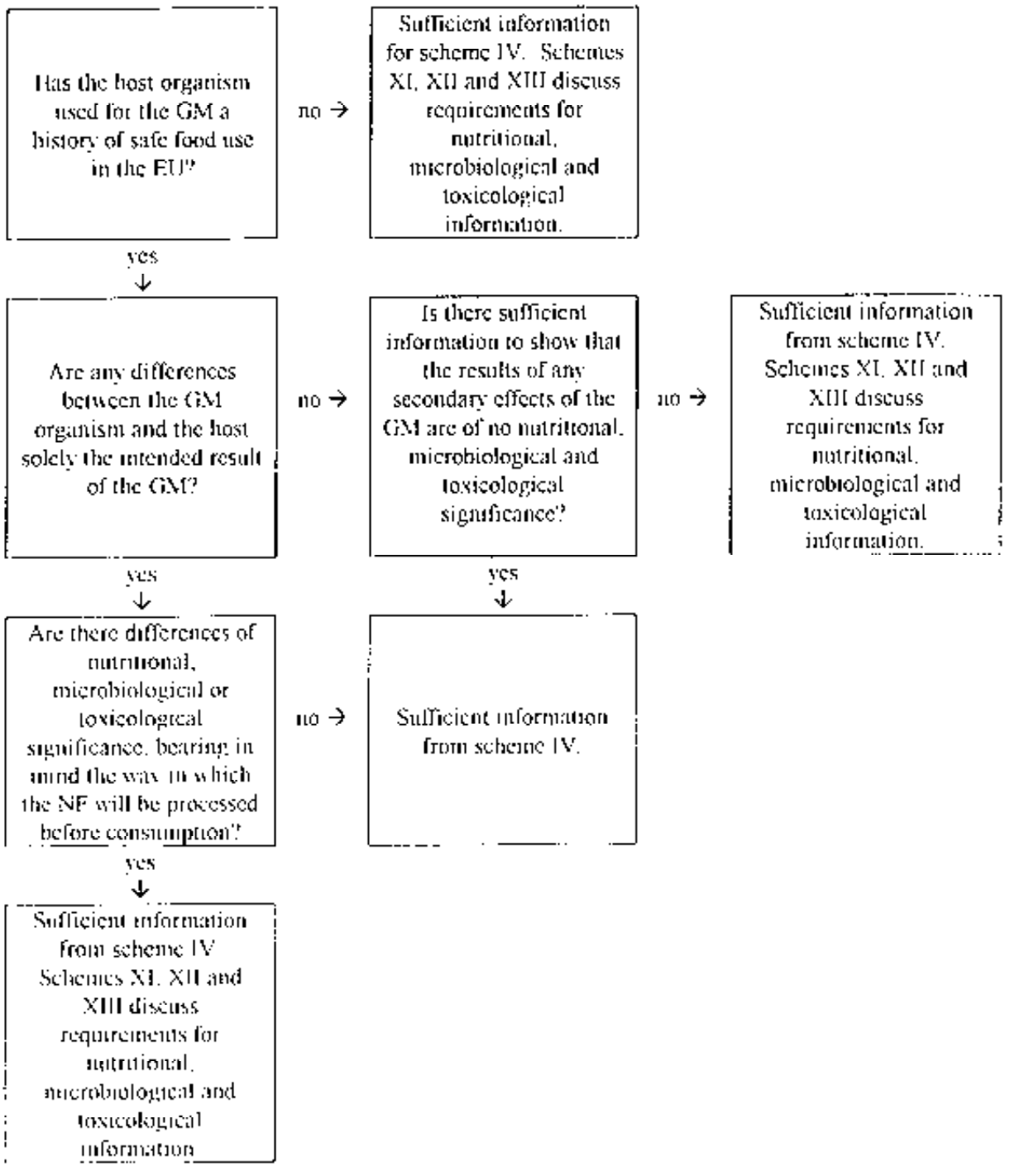
II. EFFECT OF THE PRODUCTION PROCESS APPLIED TO THE NF



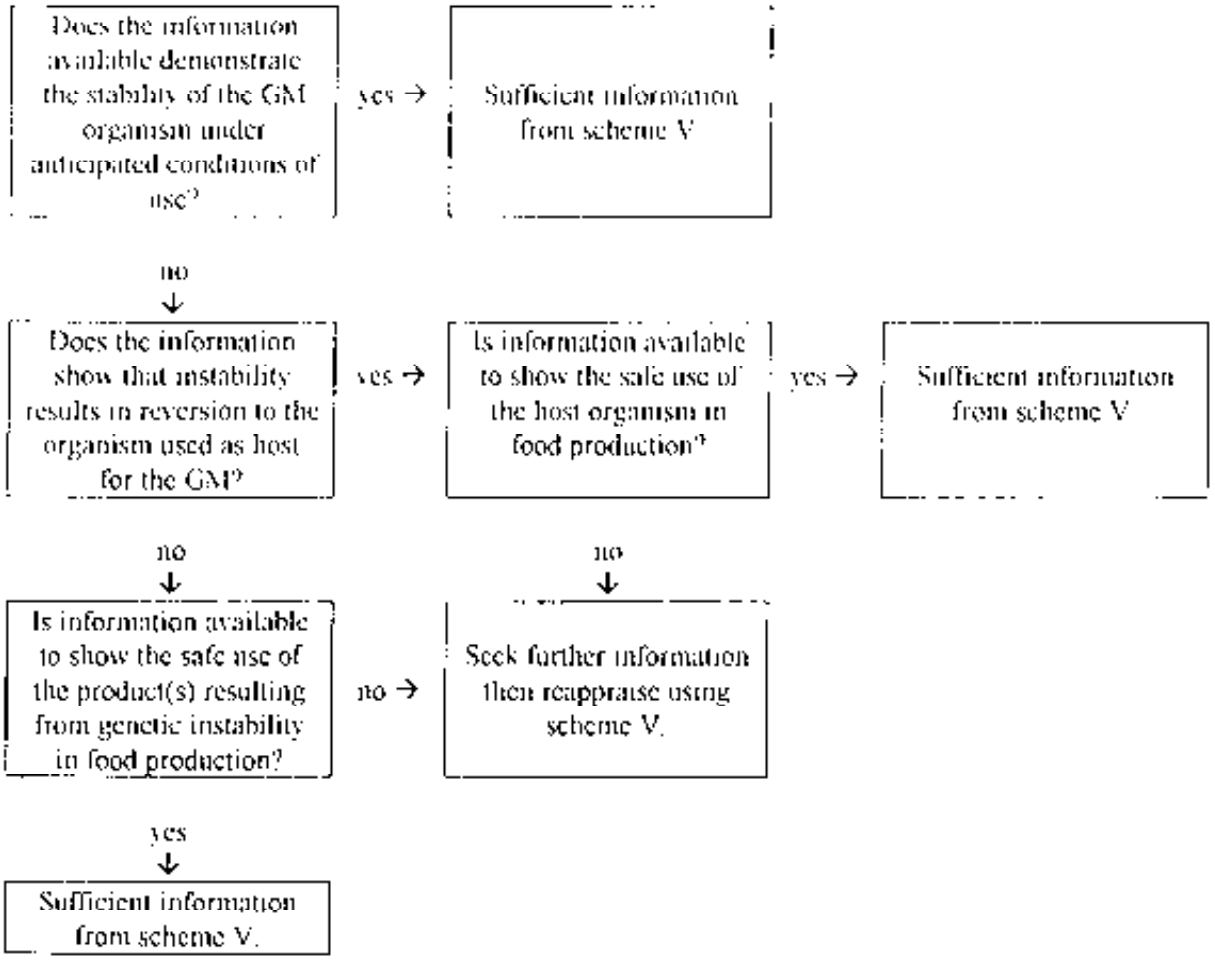
III. HISTORY OF THE ORGANISM USED AS THE SOURCE OF THE NF



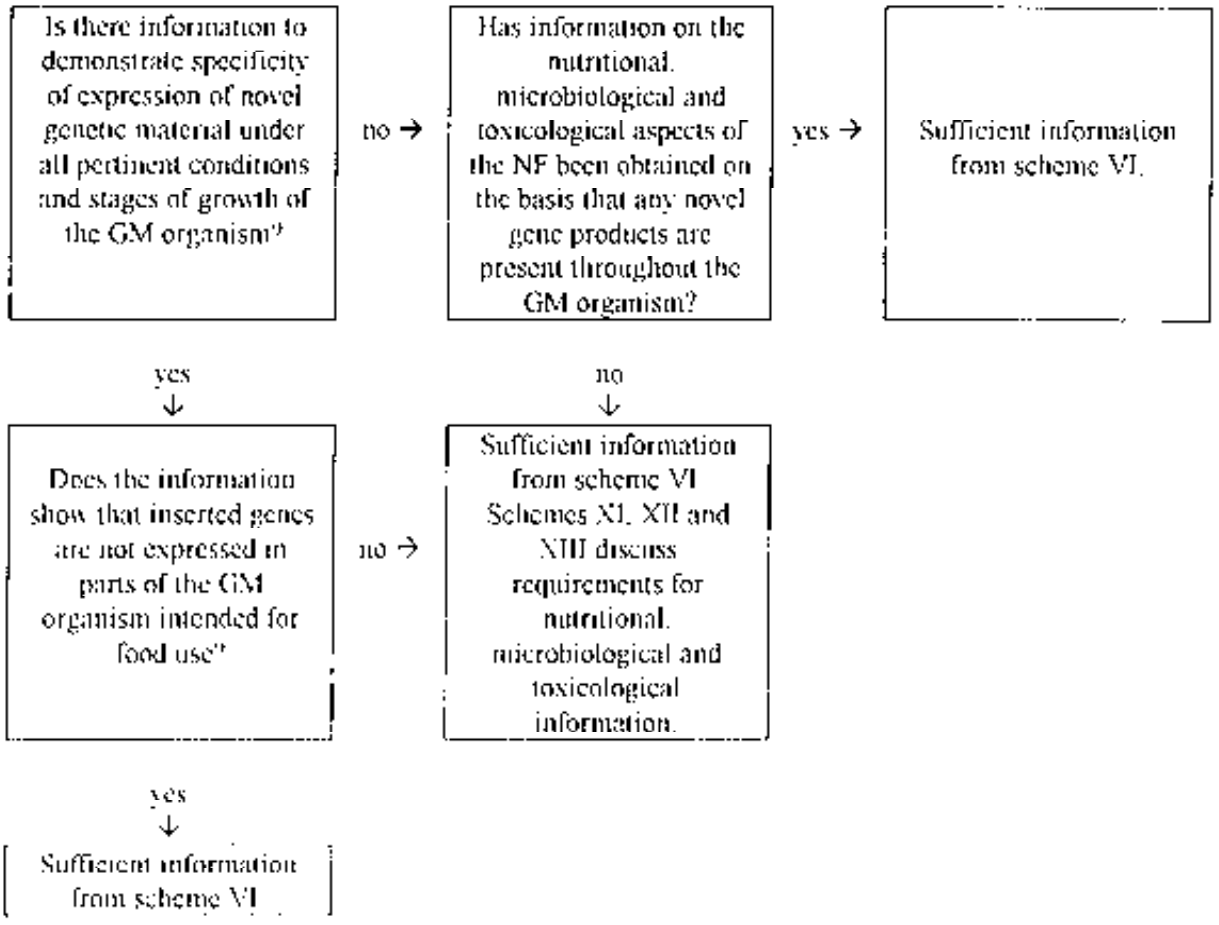
IV. EFFECT OF THE GENETIC MODIFICATION ON THE PROPERTIES OF THE HOST ORGANISM



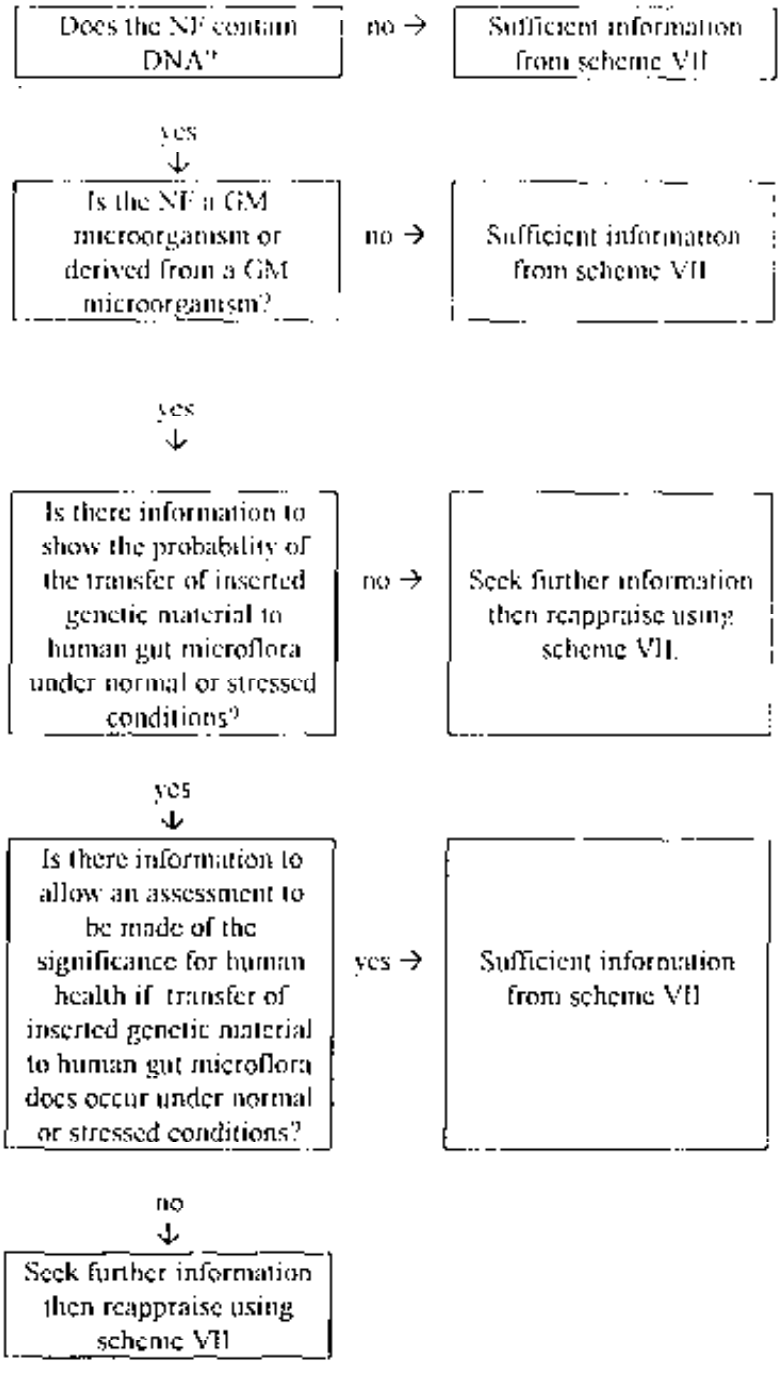
V. GENETIC STABILITY OF THE GMO USED AS NF SOURCE



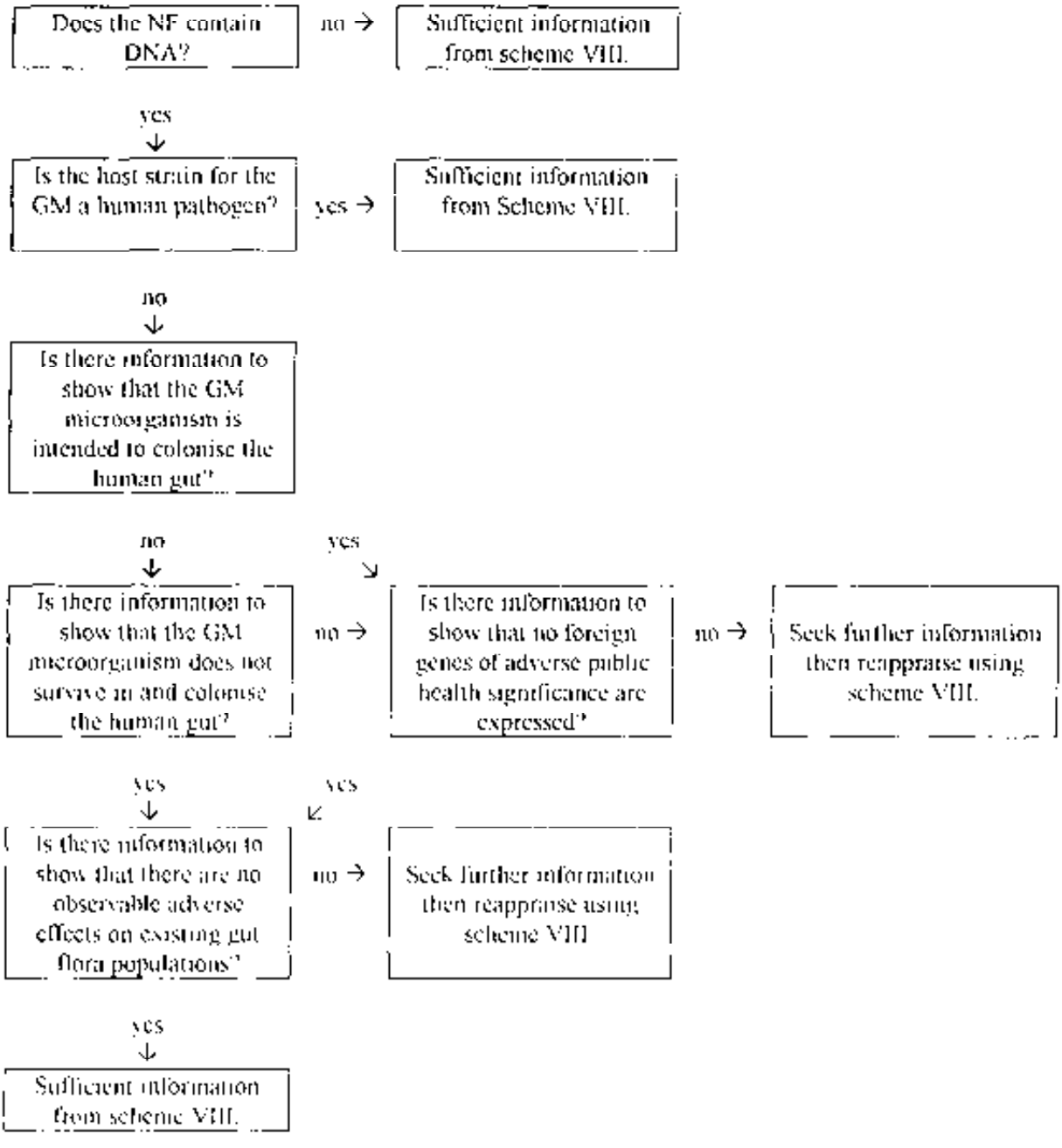
VI. SPECIFICITY OF EXPRESSION OF NOVEL GENETIC MATERIAL



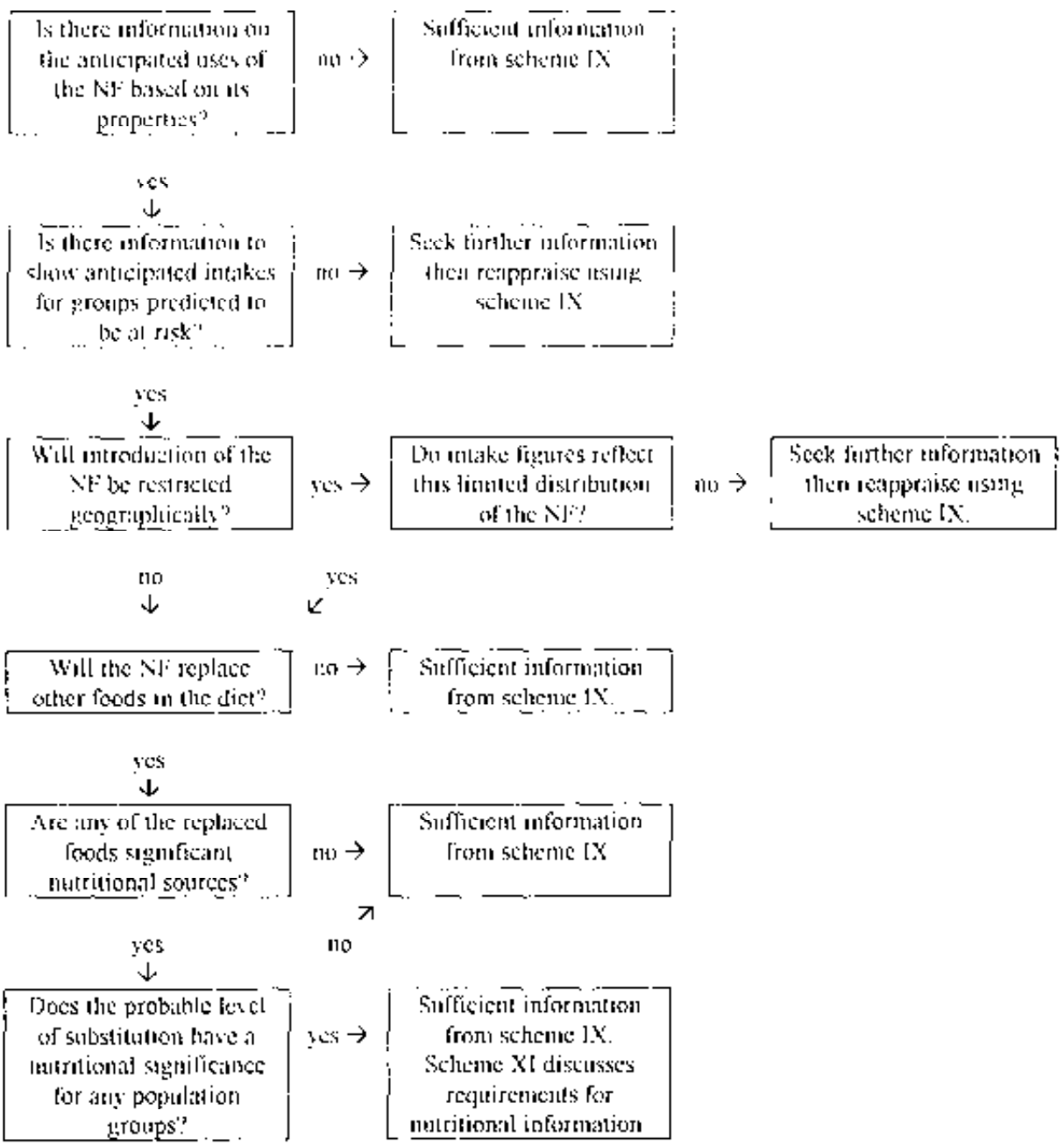
VII. TRANSFER OF GENETIC MATERIAL FROM GMO



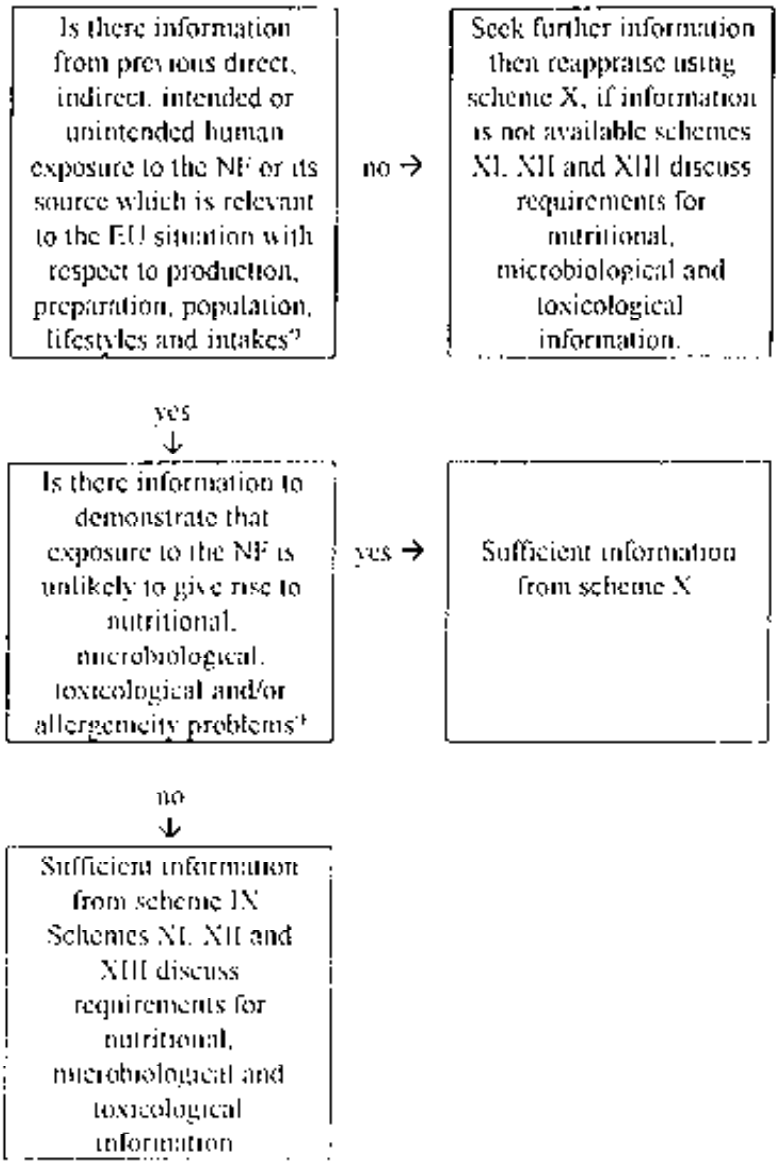
VIII. ABILITY OF THE GMM TO SURVIVE IN AND COLONISE THE HUMAN GUT



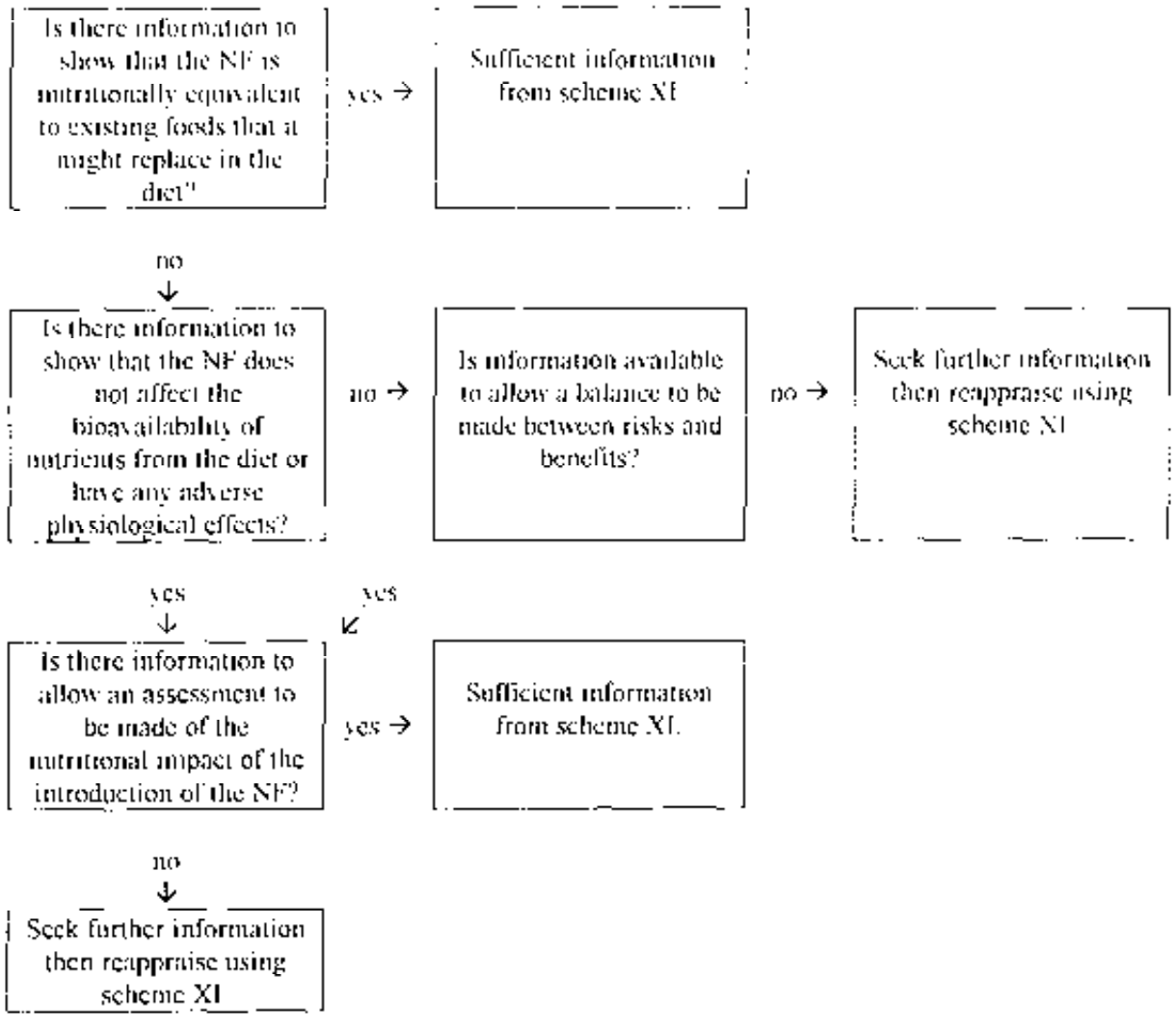
IX. ANTICIPATED INTAKE/EXTENT OF USE OF THE NF



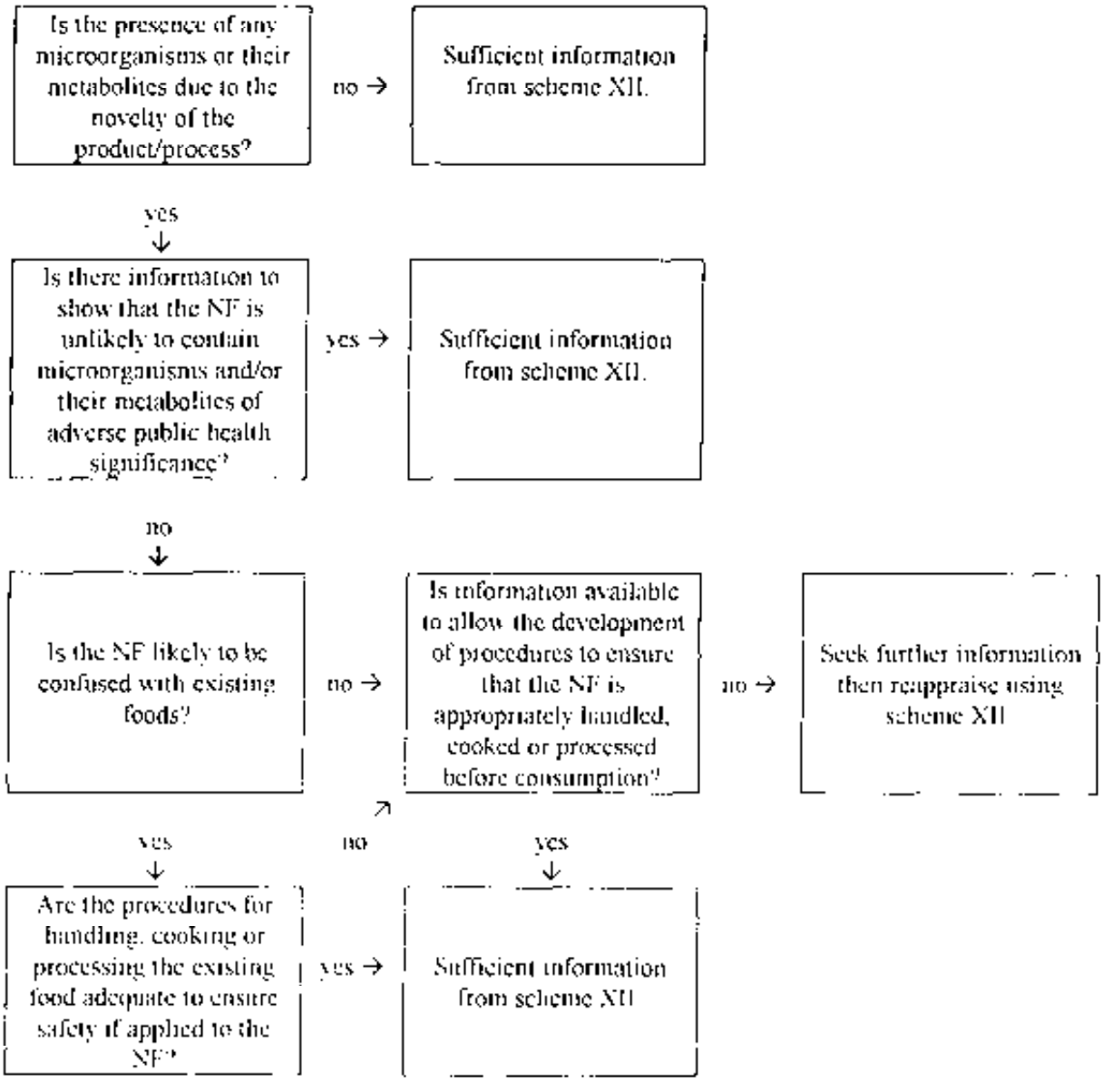
X. INFORMATION FROM PREVIOUS HUMAN EXPOSURE TO THE NF OR ITS SOURCE



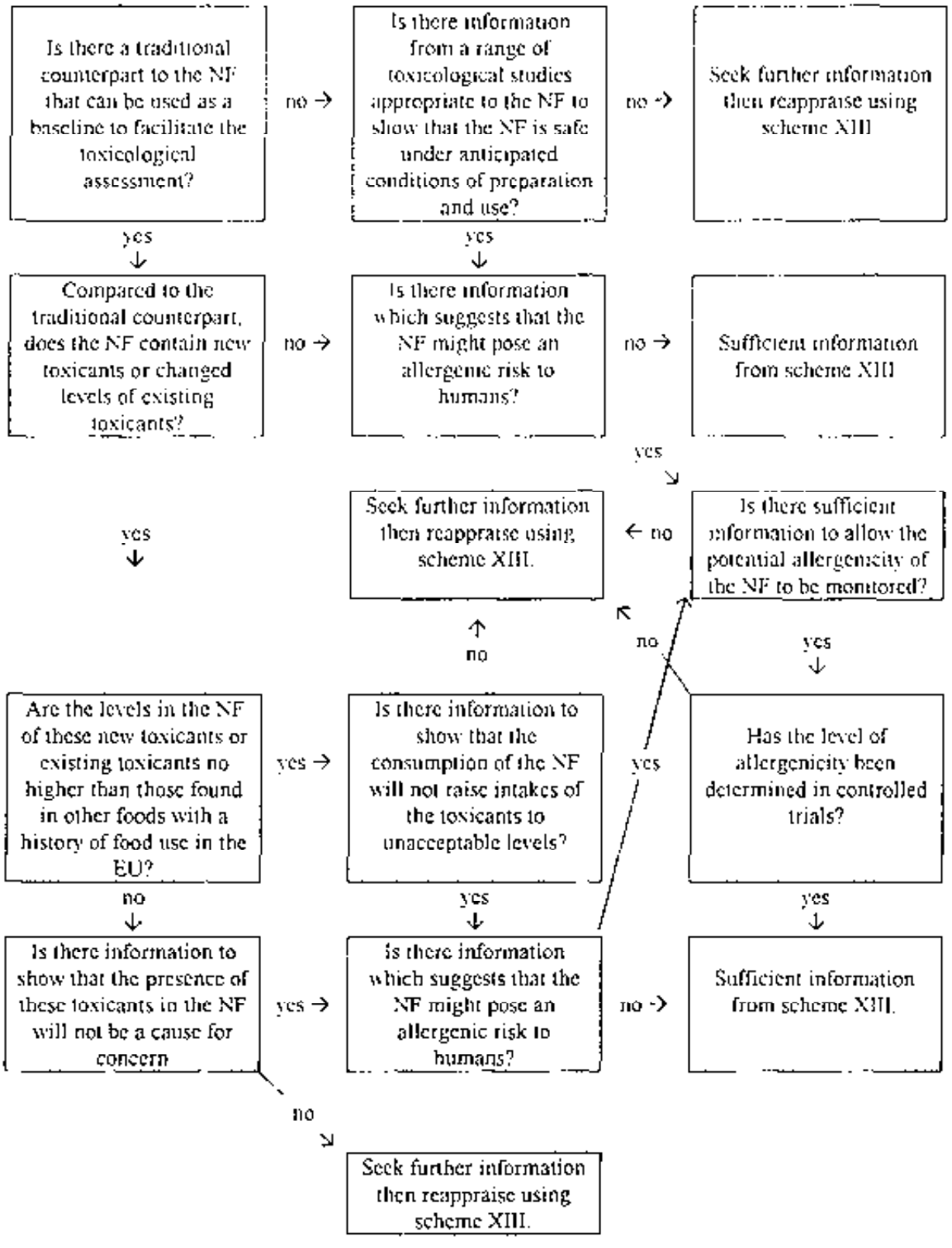
XI. NUTRITIONAL INFORMATION ON THE NF



XII. MICROBIOLOGICAL INFORMATION ON THE NF



XIII. TOXICOLOGICAL INFORMATION ON THE NF



PRINCIPLES FOR THE DEVELOPMENT OF MICROBIOLOGICAL CRITERIA FOR FOODSTUFFS AS COVERED BY THE HYGIENE OF FOODSTUFFS DIRECTIVE 93/43/EEC (1) RECOMMENDATION OF THE SCIENTIFIC COMMITTEE FOR FOODS (EXPRESSED ON 7 JUNE 1996)

Terms of reference

- To prepare for the Commission guidance on the principles to be considered when assessing the potential food hazards caused by micro-organisms and their toxins in foodstuffs and the scientific basis for the development of microbiological criteria in certain classes of foodstuffs under Article 4 of the Hygiene of Foodstuffs Directive 93/43/EEC

- To assess in accordance with such principles or guidelines adopted by the SCF the extent of the microbial hazards in foods

1 Background

1.1. This document has been produced by the Scientific Committee for Food and recommends to the Commission the scientific principles that should be taken into account when developing microbiological criteria for foodstuffs.

1.2. It describes the broad scientific framework within which consideration of the development of microbiological criteria should take place It recommends that a microbiological criterion should be based on scientific analysis and advice together with an assessment of the risk appropriate to the foodstuff and its use.**(2, 3)**

1.3. The analysis of risk from biological agents and in particular microbiological agents, is very much a developing area and as such it is recommended that further consideration is given to this within the framework of the Scientific Committee for Food, taking into account the work being undertaken by the Scientific Co-operation task on Microbiological Risk Assessment according to Directive 93/5/EEC**(4)**

1.4. The scientific principles recommended in this document can be applied generally to the development of all microbiological criteria for foods However they are specifically recommended to the Commission for application to those foodstuffs and parts of the food chain covered by the Hygiene of Foodstuffs Directive 93/43/EEC These principles are not designed or intended to be used for investigative work

1.5. The Hygiene of Foodstuffs Directive 93/43/EEC (Article 4) states that without prejudice to more specific Community rules, microbiological criteria and temperature control criteria for certain classes of foodstuffs may be adopted within the framework of the Standing Committee for Foodstuffs after consultation with the Scientific Committee for Food, following the procedure in Article 14

1.6. Fundamental to the application of this directive is the utilisation of the principles of the Hazard Analysis Critical Control Point System (HACCP) The safety of foods is principally assured through control at the source, the use of Guides to Good Hygiene Practice and the application of HACCP principles, during production, processing, handling, distribution, storage and sale

1.7. The analysis of foods for compliance with mandatory microbiological criteria must be undertaken in an official laboratory in compliance with the Official Control of Foodstuffs Directive(5), and The Additional Measures Concerning the Official Control of Foodstuffs Directive(6)

2. DEFINITION OF A MICROBIOLOGICAL CRITERION FOR FOODS

A microbiological criterion for food defines the acceptability of a process, product or food lot based on the absence or presence, or number of microorganisms, and/or quantity of their toxins/metabolites, per unit(s) of mass, volume or area.

3. COMPONENTS OF MICROBIOLOGICAL CRITERIA FOR FOODS

3.1. A microbiological criterion consists of:

- a statement of the microorganisms of concern and/or their toxins/ metabolites and the reason for that concern in the product,
- the methods for their detection and/or quantification;
- a plan defining the number of field samples to be taken, the size, and characteristics of the sample and analytical unit,
- microbiological limits considered appropriate to the food at the specified point(s) of the food chain;

- the number of analytical units that should conform to these limits;

3.2. A microbiological criterion should also state:

- the food(s) to which the criterion applies,
- the point(s) in the food chain where the criterion applies,
- the actions to be taken when the criterion is not met

4. RECOMMENDATIONS ON THE DEVELOPMENT AND APPLICATION OF MANDATORY MICROBIOLOGICAL CRITERIA FOR FOODS

4.1 The emphasis in the Hygiene of Foodstuffs Directive is placed on the use of preventive actions and the utilisation of the principles of HACCP to assure the microbiological safety of food at the point of consumption. This reduces the justification for a reliance on microbiological testing of foods for this purpose.

4.2 The development of mandatory microbiological criteria should be limited to those products and/or points of the food chain where their use is effective and the degree of protection offered to the consumer can be considered to be improved by using this type of tool

4.3 Where consideration has been given to the above and the need for a mandatory microbiological criterion has been identified, it's development should take into account as much scientific data and information as possible relating to a risk assessment The resulting criterion should be product-type specific and only applied at the point of the food chain stated

4.4. Priority for the development of mandatory microbiological criteria should be given to those micro-organisms, their toxins or metabolites in foods where a risk assessment has established a hazard to the consumer

4.5. Microbiological testing may be used by control officials and/or food businesses operators to determine the microbiological safety and wholesomeness of raw materials, ingredients, products and food lots particularly those of unknown or uncertain origin Testing can also be used to establish whether good hygienic practices have been applied and provide information on the efficacy of a business's food safety management system

4.6. In these cases, mandatory microbiological criteria may be applied to define the acceptability of raw materials, ingredients, products, food lots, and processes by control officials and/or food business operators based on an evaluation of the risk to the consumer

4.7. Depending on the results of an evaluation of the risk to the consumer the official control actions may be sorting, reprocessing, rejection or destruction of product, and/or further investigation The actions to be taken by control officials, where a microbiological limit stated in the criteria has been exceeded should be appropriate for the safety and proportionate to the risk to the consumer

4.8. Microbiological criteria used for contractual purposes by food businesses, to assess the acceptability of raw materials, ingredients, intermediate or finished products as part of their own safety management system should not be confused with legal requirements for official control purposes.

5. FACTORS TO CONSIDER WHEN ESTABLISHING AND APPLYING MICROBIOLOGICAL CRITERIA

5.1. The hygiene and safety of foods to which directive 93/43/EEC on the Hygiene of Foodstuffs applies should be ensured through the application of the requirements of the directive relating to good hygienic practices and the development and implementation of the principles of HACCP contained in Article 3

5.2. A microbiological criterion should be established and applied only where there is a definite need for it and where it can be shown to be effective and practical Such need is, for example, demonstrated by epidemiological evidence that the food under consideration may represent a public health hazard and that a criterion is meaningful for the protection of the consumer, or by the results of a risk assessment. It should be technically attainable by applying good manufacturing practice, and be realistic in terms of achievability

5.3. To fulfil the purposes of microbiological criteria, consideration should be given to:

- evidence of risk to health,
- the microbiological status of the raw material(s);
- the effect of processing on the microbiological status of the food,

- the likelihood and consequences of microbial contamination and/or growth during subsequent handling, storage and use;
- the categories of consumers concerned; and
- the cost/benefit of applying such a criteria.

5.4. The number and size of analytical units per product or food lot tested should be stated in the sampling plan and should not be modified. A product or food lot should not be subjected to repeat testing, in order to establish compliance with a microbiological criterion

5.5. The microbiological tests applied, the limits and the point in the food chain assessed should be appropriate, practical and provide as much information as possible about the safety of the food at the point of consumption

6. MICROBIOLOGICAL ASPECTS OF CRITERIA

6.1. Microorganisms and toxins of importance in a particular food.

6.1.1. For the purpose of this document microorganisms and toxins include.

- bacteria, viruses, yeasts, and moulds and algae.
- parasitic protozoa,[4]
- their toxins/metabolites.

6.1.2. The microorganisms included in a criteria should be widely accepted as relevant as pathogens or as indicator organisms to the particular food and food business operation

6.1.3. The mere finding, with a presence or absence test of certain organisms known to cause foodborne illness may not necessarily indicate a hazard, the relevance of their presence should relate to the results of the risk assessment

6.1.4. Where pathogens can be detected directly and reliably consideration should be given to testing for them in preference to indicator organisms. If a test for an indicator organism is applied there should be a clear statement as to whether the test is used to indicate an unsatisfactory hygiene practice or the possible presence of a health hazard

[4] Helminths may be considered in specifications when appropriate

6.2. Microbiological methods.

6.2.1. Preference should be given to reference methods developed under the aegis of an European Standards Institute which have already been validated for the commodity concerned. When this is not possible, only methods for which the reliability (accuracy, reproducibility, inter- and intra-laboratory variation) has been statistically established in comparative or collaborative studies in several laboratories should be used in the microbiological criterion.

6.2.2. Methods used to determine the suitability for consumption of highly perishable foods, or foods with a short shelf-life, should be chosen such that the results of microbiological examinations are available before the foods are consumed or exceed their shelf-life.

6.2.3. The microbiological methods specified should be reasonable with regard to complexity, availability, ease of interpretation, time required and costs.

6.2.4. Methods which are applicable to various groups of commodities should be given preference over methods which apply only to individual commodities.

6.3. Microbiological limits.

6.3.1. Limits used in criteria should be based on microbiological data appropriate to the food and should be applicable to a variety of similar products. They should therefore be based on data gathered over as wide a range of situations as possible and where good hygienic practices are in operation.

6.3.2. In the establishment of microbiological limits, changes in the microflora which could result from likely storage and distribution practices should be taken into account. For the development of mandatory microbiological criteria within the terms of directive 93/43/EEC, when appropriate, the conditions under which the food is expected to be handled and consumed.

6.3.3. Numerical limits should also take account of the likelihood of uneven distribution of microorganisms in the food and the inherent variability of the analytical procedure.

6.3.4. It should be borne in mind that no feasible sampling plan can ensure complete absence of a particular organism in a product or food lot.

7. SAMPLING PLANS, METHODS AND HANDLING

7.1. A sampling plan is the particular choice of a sampling procedure and the decision criteria to be applied to a lot, based on examination of a prescribed number of analytical sample units by defined methods. Sampling plans should be administratively and economically feasible.

In particular, sampling plans should take into account:

- consideration of the severity of the hazard and an assessment of the risk
- the heterogeneity of distribution of microorganisms,
- the statistical probability of detecting unacceptable food lots, or rejecting acceptable food lots

For many applications 2- or 3-class attribute plans may prove useful (7)

7.2. The sampling method should be defined in the sampling plan, the time between taking the field samples and analysis should be as short as possible. During transport to the laboratory the conditions (e.g. temperature) should be appropriate to the food, so that the results reflect - within the limitations given by the sampling plan - the microbiological conditions of the lot, or food product.

8. REPORTING

8.1. The test report shall give the information needed for complete identification of the sample, the sampling plan, the test methods, the results, and their interpretation.

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European Commission

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The Scientific Committee for Food was established by Commission Decision 74/234/EEC of 16 April 1974 (OJ L 136, 20.5.1974, p. 1), replaced by Commission Decision 95/273/EC of 6 July 1995 (OJ L 167, 18.7.1995, p. 22), to advise the Commission on any problem relating to the protection of the health and safety of persons arising or likely to arise from the consumption of food, in particular on nutritional, hygienic and toxicological issues.

The members are independent persons, highly qualified in the fields associated with medicine, nutrition, toxicology, biology, chemistry, or other similar disciplines.

Responsibility for the secretariat of the Scientific Committee for Food was transferred from Directorate General III 'Industry' to Directorate-General XXIV 'Consumer Policy and Consumer Health Protection' with effect from 1 April 1997.

The present report deals with opinions on:

- The scientific basis of the concept of threshold of regulation in relation to food contact materials;
- Products derived from bovine tissues, especially gelatin, tallow and di-calcium-phosphate in relation to bovine spongiform encephalopathy;
- The use of ozone for the removal of unstable elements such as iron, manganese and arsenic from natural mineral water;
- Dimethyldicarbonate (DMDC, Velcorin);
- Phthalates in infant formulas;
- The calculation of vitamin E content in infant formulas and follow-on formulas;
- Assessment of novel foods
  Part I: Recommendations concerning the scientific aspects of information necessary to support applications for placing on the market of novel foods and novel food ingredients;
- Report on:
  Principles for the development of microbiological criteria for foodstuffs as covered by the hygiene of foodstuffs Directive 93/43/EEC — recommendation