Toxicological Modes of Action: Relevance for Human Risk Assessment

Technical Report No. 99

ISSN-0773-8072-99
Brussels, July 2006

ECETOC TECHNICAL REPORT No. 99

European Centre for Ecotoxicology and Toxicology of Chemicals
4 Avenue E. Van Nieuwenhuyse (Bte 6), B-1160 Brussels, Belgium.

Toxicological Modes of Action: Relevance for Human Risk Assessment

CONTENTS

EXECUTIVE SUMMARY

Generally, the assessment of hazard and risk to humans that might arise from exposure to chemical substances is based on the extrapolation from data generated in studies with experimental animals. The assumption is made that the effects observed in the animals would also be expressed in humans and, to account for uncertainties including intra- and interspecies variability, and more recently susceptible human subpopulations, assessment factors are applied in deriving 'safe' exposures for humans.

This is usually done without testing the hypothesis in humans, as the default approach allows for humans to be more sensitive than the most susceptible animal species. However, for those substances where humans are not sensitive to the effects seen in the animal studies, this approach is conservative and may result in risk management measures being taken that are totally unwarranted. An alternative approach is proposed in this report for the evaluation of such substances.

The view has been expressed by many leading researchers and regulatory organisations that an understanding of the molecular and cellular processes underlying toxicity and carcinogenicity allows a more scientifically-based risk assessment and, where such information is available and adequate, this should replace default approaches in assessing human risk.

All mechanisms of toxicity can be described in terms of a sequence of events, each of which is critical to the manifestation of the toxic endpoint. To elucidate such a mechanism requires rigorous investigation to obtain a complete and detailed understanding of the process leading to a toxic effect. However, the complete elucidation of all the events leading to the effect is not essential for evaluating the species differences in sensitivity to a toxicant. To achieve this we can restrict our understanding to selected key events within the complete sequence of events. This concept, termed the 'mode of action' can be used for the evaluation of species specificity including human susceptibility. If it could be shown that one or more of the key events could not occur in humans, or could only occur to a much lesser extent, it could be assumed that the mode of action of the substance in question was not relevant to the assessment of human risk. In such cases departure from the default approach would be justified.

This philosophy is illustrated by a number of well-studied examples for which the modes of action and corresponding key events have been established for effects that are not relevant to humans.

A structured two-step 'mode of action' approach was developed to guide the evaluation of substances expressing toxicity suspected as being species-specific and of questionable relevance to humans. Advice is given on how to identify the key events in the mode of action of the

toxicological effect, how to test the strength of the data on which the mode of action hypothesis is based and how to establish its relevance to humans.

The extent to which new data will be required to associate a substance with an established mechanism of toxicity or mode of action will vary, depending on the existing knowledge of the role of toxicokinetics and toxicodynamics and of the understanding already available within that class of chemicals.

The use of this approach to identify those substances which express toxicity in experimental animals via a mode of action that is not relevant to humans will enable a more scientifically objective assessment of risk in humans. Furthermore, adopting a 'mode of action' approach should considerably speed up the risk assessment process and avoid unnecessary animal experimentation.

1. INTRODUCTION

1.1 Background

Fundamental to the evaluation of the safety of chemicals is an understanding of their intrinsic toxicological properties. Commonly, such data are derived from toxicological studies carried out to comply with requirements of national and international regulatory organisations such as the US EPA, JMAFF and EC. In general observations are made in laboratory animals exposed to a range of dosages or concentrations by the most appropriate route, or routes, of administration taking into account likely human exposure patterns. The inclusion of more than one species can provide reassurance that a critical effect for humans will not be missed by studying only a potentially insensitive species. The data generated in such studies are used in two ways. Firstly, to determine the potential hazard of the substance by taking into account the route of exposure and nature of any observed adverse effects; secondly, to gain an understanding of the potential risks to man by comparing the dose at which these effects occur with known or estimated human exposures. The critical determining factors in the second step will be the adverse effect(s) for which the protection of the exposed population is required and the exposure level(s) at which such effects do and do not occur.

In the absence of comparative data in humans, simple and conservative 'worst case' assumptions are made, the most fundamental being that all adverse effects of a substance that are observed in animal studies, are relevant for humans. Where more than one adverse effect arises from exposure to the substance under evaluation, the assessment of hazard and risk to humans is generally based on the 'lead' effect (toxic effect of concern), i.e. the most serious effect evoked at the lowest exposure level. Where data from more than one species or strain are available, the assessment is based on the most susceptible of these.

In assessing the risk to humans, this conservative procedure is reinforced through the application of 'safety', 'assessment' or 'uncertainty' factors to take account of uncertainty over interspecies and intraspecies differences in sensitivity to the test substance (WHO, 1987; Renwick and Lazarus, 1998). Further details and guidance on the derivation and application of appropriate assessment factors in human health risk assessment can be found in ECETOC Technical Report No 86 (ECETOC, 2003).

For many substances the default approach provides adequate margins of safety for the potentially exposed population for the defined use of the chemical. However, in some situations, e.g. where humans are demonstrably much less sensitive than the test species or, indeed, where it is known that the effects seen in the test animal would under no circumstances be manifest in humans, such conservatism could lead to risk management measures that are unnecessary.

There are a growing number of chemicals where application of the usual default approach to risk assessment has been shown to be too conservative; many of these cases have been recognised formally by regulators and by international organisations.

Scientists from CIIT were among the first to publish a strategy in which risk assessment approaches for animal carcinogens were founded on an understanding of their modes of action (Butterworth *et al*, 1995). In 1996 and 1999, US EPA included the use of mechanistic data in their 'Proposed Guideline for Carcinogenic Risk Assessment' (US EPA, 1996, 1999). Meanwhile the application of mechanistic / mode of action data was further recommended and extended to the assessment of non-cancer endpoints (Schlosser and Bogdanffy, 1999; Haber *et al*, 2001; Sonich-Mullin *et al*, 2001).

A common theme of these proposals is that an understanding of the molecular and cellular processes underlying toxicity and carcinogenicity allows a more scientifically-based risk assessment and, where such information is available and adequate, this should replace default approaches in assessing human risk.

Against this background a Task Force was commissioned by ECETOC to examine, in the context of human risk assessment, the role and use of mode of action and mechanistic data in establishing the relevance of toxicological effects observed in experimental animals.

The Task Force was assigned the following Terms of Reference:

- Illustrate, with examples, how it can be demonstrated that a mode of action observed in an animal model is not relevant, or cannot be extrapolated to humans.
- Provide a rational scientific approach by which it might be judged to what extent a mode of action seen in an animal model can be considered not relevant for humans.

1.2 Scope of report

Conventionally, justification for excluding positive toxicity data on the grounds of non-relevance to humans requires extensive mechanistic data that provide a complete and detailed understanding of all the steps in the sequence of events leading to the toxic effect. There are relatively few substances for which such comprehensive toxicological data exist. However, the complete elucidation of all the events preceding toxicity is not believed to be essential to establish major differences in sensitivity between species.

Two main aspects of toxicity, toxicokinetics and toxicodynamics, can account for the nature and extent of differences between species in their sensitivity to substances. The implications for risk assessment are considered in the opening chapters of the report.

The expression of toxicity in a mammalian system is dependent on a sequence of key events taking place, each of which is critical to the manifestation of the toxic endpoint. Thus, for a specific toxic endpoint, to justify deviating from the default approach in risk assessment, it should be sufficient to identify the key events in the process (mode of action) and establish that these would not occur in humans, or that they would occur only to a much lesser extent. From such knowledge it could be concluded that the toxic outcome would not be observed in humans, or be observed only at much higher and possibly irrelevant exposure levels. A proposal is made, illustrated with well studied examples, for a structured approach for establishing and reviewing the mode of action by which a substance causes a toxicological effect. This allows a decision to be made on the appropriate animal endpoint for human hazard identification and risk assessment. This concept is elaborated in the context of extrapolation of toxicity data from animals to humans, and guidance given on testing the hypothesis on which the non-relevance to humans is based.

Throughout this report, a clear distinction is made between two terms often used in this context, i.e. ‘mechanism of toxicity’ and ‘mode of action’ of a substance. Identifying a ‘mechanism of toxicity’ requires rigorous investigation to obtain a comprehensive understanding of the entire sequence of events that result in the toxic effect of interest. In practice, ‘mechanisms of toxicity’ are rarely established. For the ‘mode of action’ concept it is sufficient to develop an understanding of key events within the complete sequence of events leading to toxicity. This does not require the amount or depth of data needed to provide a detailed explanation of the complete sequence of events.

This document is intended as a reference for industrial, academic and regulatory toxicologists, as well as those individuals less familiar with the evaluation process, to aid the formulation and application of consistent judgements in the interpretation of toxicity data and their extrapolation to humans. This initiative is one of three component parts of a project aimed at providing guidance on the process of human risk assessment of chemical substances. The two other parts looked at adverse vs. non-adverse effects in toxicological studies, published as Technical Report No. 85 (ECETOC 2002) and risk assessment factors for human health risk assessment, published as Technical Report No. 86 (ECETOC 2003).

2. DIFFERENCES IN SENSITIVITY BETWEEN ANIMALS AND HUMANS

The expression of toxicity arising from exposure to a chemical substance is a consequence of a chain of events that results in the affected tissues of an organism receiving a sufficient quantity of that substance (or its ultimate toxic metabolites) to produce the adverse effect. The factors that confer species susceptibility, and lead to major differences between animals and humans in their response to such chemical insults, are those that modulate these critical requirements, either in the nature and quantity of the ultimate toxicant that is presented to the sensitive tissues (toxicokinetics) or in the sensitivity of those tissues to the ultimate toxicant, i.e. the toxicodynamic response.

2.1 Toxicokinetic differences

The toxicokinetic phase begins with exposure and results in a certain concentration of the ultimate toxicant at the target site. This concentration is dependent on the absorption, distribution, metabolism and excretion (ADME) of the substance. Species differences are known to occur at each stage within this sequence, leading to different target site concentrations of the parent compound or its critical metabolites.

There are several well-investigated substances where toxicity is determined by different species-specific toxicokinetic profiles. In the case of a major quantitative or even a qualitative species difference, a toxic metabolite might be present only at low concentrations or even below the limit of detection in the non-responsive species. In other cases, moderate or minor quantitative differences explain quantitative differences in toxic response.

Coumarin and methylene chloride are examples of substances where tumour formation appears to be the consequence of metabolic activation that is specific to rodents. The particular susceptibility of dogs to adverse effects of phenoxyacetic acids and related compounds results from a markedly lesser ability of this species (compared to others) to excrete organic acids. These examples are well documented in the literature and are summarised in Appendix A.

2.2 Toxicodynamic differences

In the toxicokinetic phase (Section 2.1) the bioavailability of the substance or its toxic metabolite(s) characterises the species difference in response. The toxicodynamic phase comprises all events, subsequent to the delivery of the ultimate toxicant to the target tissues, which finally lead to the toxicological effect; these include interaction of the substance / toxic metabolite(s) with the target tissues (comprising cells, organelles and biological macromolecules

such as enzymes, receptors and DNA) and all patho-physiological consequences thereof, ultimately leading to the expression of toxicity. A species difference in response can arise from a difference in the interaction of a substance with the target, e.g. where the respective molecular target is absent in the non-susceptible species. Even if the target is present and the substance does interact, the biochemical and/or patho-physiological consequences may be different in different species.

There are several well-investigated examples where species-specific toxicity is explained either by qualitative or by major quantitative differences at the target site. Two such examples are α2u-globulin nephropathy in the male rat, and the development of tumours of the forestomach in rodents; the target protein and the target tissue, respectively, are not present in humans. In other examples, such as the formation of thyroid follicular cell tumours by liver enzyme inducers in the rat or triketone-mediated tyrosinaemia leading to corneal opacity in the rat, the arguments for non-relevance for humans rely on cellular and/or molecular aspects, as well as on knowledge from human aetiology and epidemiology (see Section 4, and Appendix B for further details of these and other examples).

2.3 Implications of toxicokinetic and toxicodynamic species differences

In the examples quoted in Section 4 and Appendices A and B, the weight of evidence demonstrates clearly that a chemically induced toxic effect in an animal species is unlikely to occur in humans. This lower or even absence of susceptibility in humans is the result of a fundamental difference between animals and humans in either ADME or in the toxicodynamic effect of the substance and its biotransformation products.

Where the species difference is due to differences in bioavailability (e.g. metabolic activation of methylene chloride uniquely in mice), the arguments in favour of non-relevance for humans are substance-specific. However, where there is a difference in toxicodynamics and the established mode of action is unique for the species and sex (e.g. chemically induced α2u-globulin nephropathy and kidney tumours in the male rat), arguments in favour of non-relevance to humans are species- / sex-specific. Thus, where any substance is shown to be nephrotoxic and causes kidney tumours only in the male rat, it can be concluded that such a finding is not relevant in the assessment of the human risk of that substance, provided that it can be shown that the mode of action is *via* the formation of α2u-globulin.

In some cases, the establishment of non-relevance to humans is possible only with quantitative analysis of extensive data on a range of parameters. One such example is formaldehyde where target tissue dosimetry (quantification of DNA-protein cross-links) together with species differences in physiological parameters, were analysed using physiologically based kinetic modelling; this enabled the derivation of more appropriate risk estimates for humans in place of application of the default assessment factors.

3. EXTRAPOLATION OF A TOXIC EFFECT OBSERVED IN ANIMALS TO HUMANS

The potential risk to human health from exposure to chemical substances is generally assessed by extrapolation from animal data to humans. Two approaches can be used for such extrapolation. In the first, which is the default approach, the assumption is simply made that all effects observed in animals are relevant to humans. To account for interspecies and intraspecies differences in sensitivity to the substance, numerical factors are used to establish safe levels of exposure for humans. The derivation of such generic assessment factors and their application in human health risk assessment is the subject of Technical Report No. 86 (ECETOC, 2003) and is not discussed further in this report.

If the available data suggest that the effect observed is of questionable relevance to humans, a second approach may be appropriate in which the mode of action underlying the effect is examined and taken into account to justify departure from the first (default) approach.
In the following sections this second approach is presented in detail.

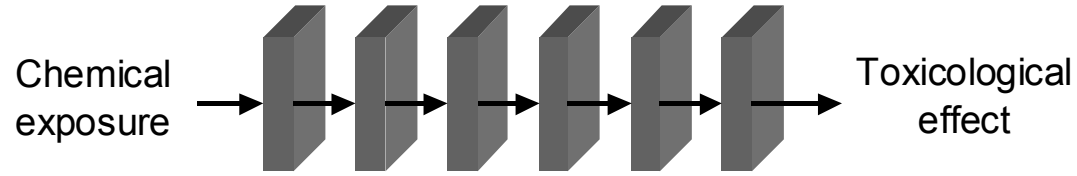
3.1 Identification of mode of action

The first stage in the process of extrapolating a toxic effect observed in animals to humans is the identification of the mode of action that results in the adverse effect(s) observed in the susceptible species. If the mode of action is unknown, the test organism can be considered as a 'black box', i.e. a system that, on exposure to a sufficiently high amount of the substance, responds with the effect (Figure 3-1).

Figure 3-1: Black box (1)

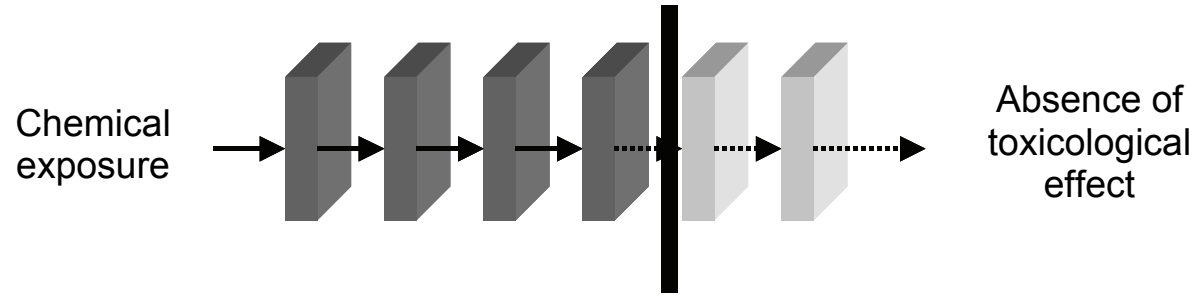
A sequence of key molecular and cellular events takes place within the black box, beginning with exposure to the substance and ending with the expression of toxicity (Figure 3-2). Each key event in the process can be assigned either to the toxicokinetic or to the toxicodynamic phase.

Figure 3-2: Black box (2)



The concept of describing the entire process from exposure to toxicological effect as a sequence of key events implies that the effect will be observed only under conditions where each of these key events within the sequence proceeds to a sufficient extent. In other words, if within this sequence of key events, one critical event does not take place, or does not take place to a sufficient extent, the ultimate toxicological effect will not be observed (Figure 3-3).

Figure 3-3: Black box (3)



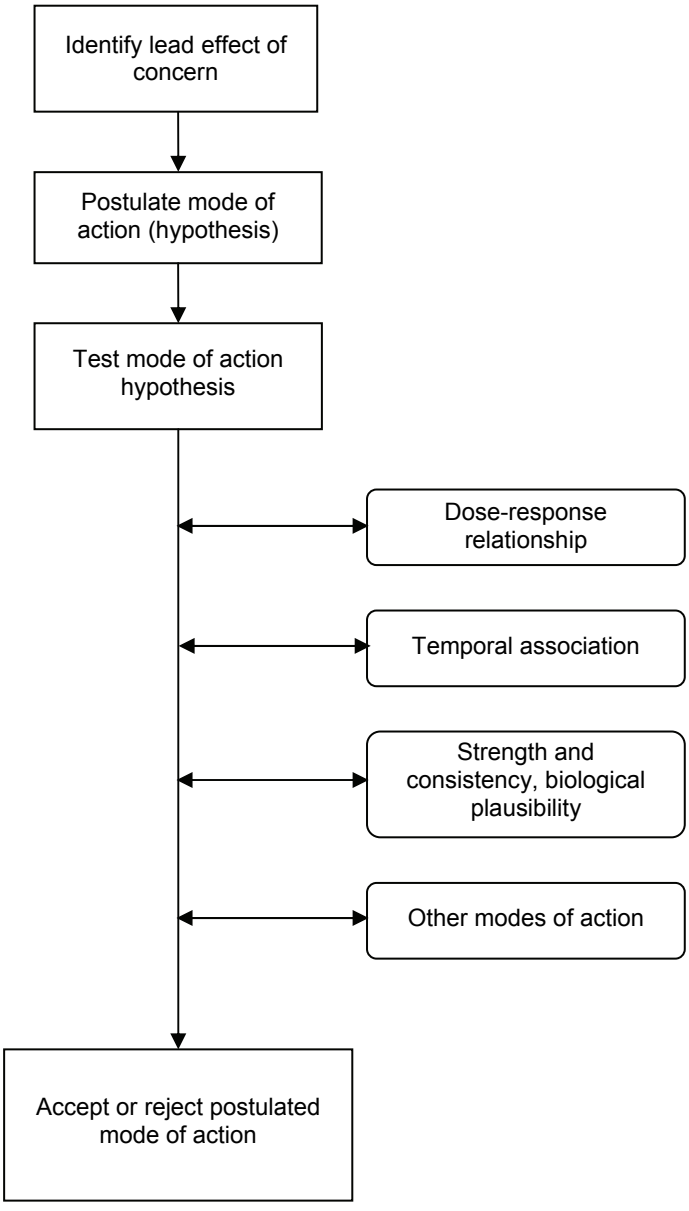
The phenomenon of species differences in toxicity can be examined in the context of this process. Within this concept, any species difference must be the consequence of a difference in the response to at least one of the key events.

3.2 Postulation and testing of a mode of action hypothesis

The adoption, or rejection, of a proposed mode of action is based on the toxicity data available for the substance. The major part of such data generally consists of studies performed according to recognised regulatory guidelines. In addition, relevant substance-specific information may be available from toxicokinetic and metabolic studies and from specially designed *in vivo* and/or *in vitro* mechanistic or mode of action studies. This can be supplemented by data from structurally-related substances and from substances with similar toxicological profiles. Human data for the substance or related substances are highly relevant and where available, should be included in the assessment.

The conclusion as to the strength of the data in support of the postulated mode of action (Scheme 3-1) is reached essentially by following the 'Hill Criteria' (Hill, 1965), adapted for mode of action analysis in experimental toxicology (Schlosser and Bogdanffy, 1999) and cancer risk assessment (Sonich-Mullin *et al*, 2001. These principles are described in the following sub-sections.

Scheme 3-1



3.2.1 Proposed mode of action

The key events in the proposed mode of action should be defined clearly and any species differences highlighted in the expression of the toxic effect of concern. Such key events should be quantifiable.

3.2.2 Dose-response relationship

In relation to both the parent substance and the ultimate toxicant, a comparison of dose-response information in susceptible animals is critical to support or invalidate the proposed mode of action. For example, for a substance that requires metabolic activation, the variation in the production or removal of the ultimate toxicant is critical to the difference in the toxicological response of the respective species.

If a key event is postulated to be critical to the mode of action, it should be observed at all dose levels, including the lowest, at which the ultimate toxic effect is expressed.

3.2.3 Temporal association

The key events that are essential for the expression of toxicity must occur in a plausible sequence that is consistent with the proposed mode of action.

Postulated key events should be observed after shorter treatment periods than those that cause the ultimate toxic effect.

3.2.4 Strength, consistency and biological plausibility

The more extensively a hypothesis is challenged experimentally, the greater will be the confidence in the postulated mode of action. Both direct and indirect evidence can be of value in ascertaining the strength and consistency of the proposed mode of action.

Modulation of the toxicological effect can be a useful experimental challenge of a mode of action hypothesis. For example, the blocking of a key event, such as metabolic activation, should result in significant diminution of the toxicological effect. In contrast, induction of the metabolic step should increase the extent of bioactivation and hence the toxicological response.

The proposed mode of action should not conflict with generally accepted facts or principles relating to the pathogenesis of the ultimate toxicological effect and should be internally consistent

with other aspects of the toxicology dataset for that substance. If the mode of action is relevant to a family of structurally-related substances, then data on the toxic effects of other family members would be expected to complement and strengthen the existing data on the substance in question.

3.2.5 Eliminating other modes of action

The strengths, plausibility and coherence of alternative modes of action that might result in the same toxicological effect should be examined. For example, if tumour formation was the toxic endpoint and a non-genotoxic mode of action was postulated, data should be available that support the exclusion of genotoxicity as the mode of action.

3.2.6 Outcome of action assessment

The outcome of the assessment might be that the existing data provide a convincing or reasonable argument in favour of the postulated mode of action.

In cases where the data leave important questions unanswered, or where the data raise some inconsistencies, additional information will be needed before the postulated mode of action can be accepted.

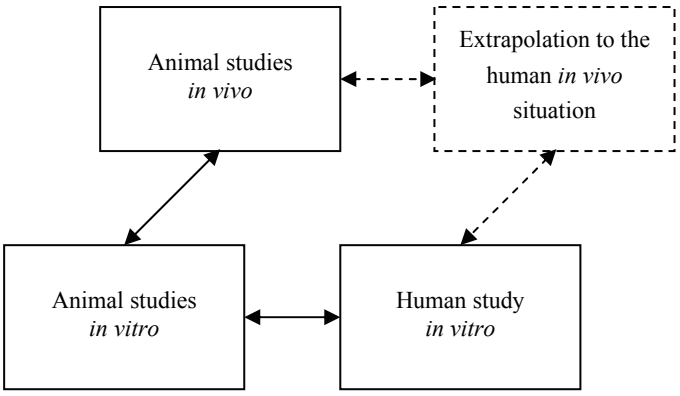
3.3 Assessment of human relevance

Once a mode of action has been established for the lead effect in experimental animals, its relevance to humans should be assessed. The human relevance of the effect can be challenged if at least one identified key event does not take place, or does not take place to a sufficient extent, in humans. Such a key event might be identified at any point in the sequence of events that takes place between exposure to the substance and expression of the toxic effect.

To demonstrate the existence of such a key event is challenging, particularly for substances where the results of testing in humans are not available or appropriate. A straightforward example is where a specific toxicological effect is not observed in all the animal species investigated. This species difference provides relevant 'strength and consistency' arguments in favour of a postulated mode of action. The subsequent assessment of the human relevance of the effect can then be directed at discovering whether the human is similar to the susceptible or to the non-susceptible animal species. This can only be addressed by additional investigations, such as *in vitro* studies with human tissues. This means that once the mode of action in experimental animals is understood, an *in vitro* model system (cellular / sub-cellular) correctly predicting the observed response in susceptible and non-susceptible animals *in vivo*, can be established. As

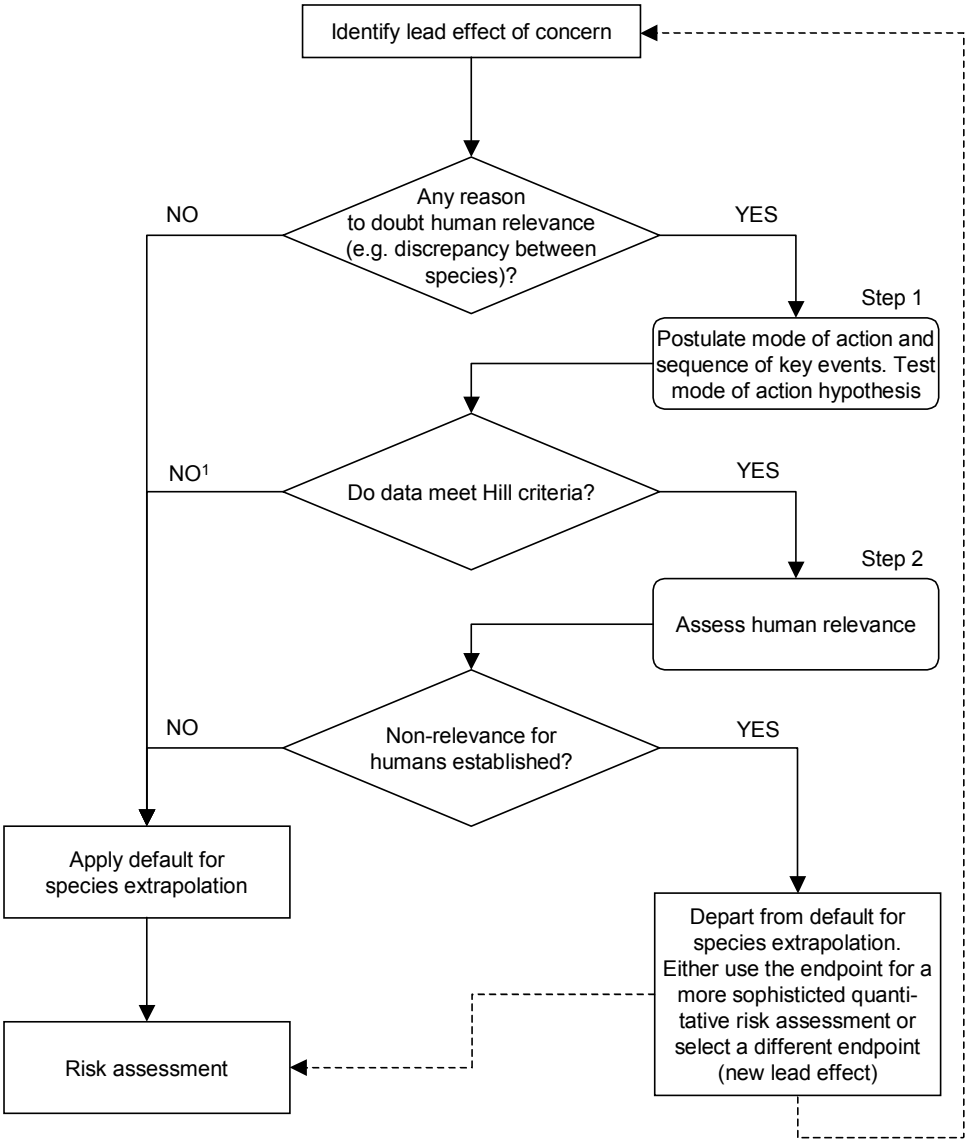
illustrated in Scheme 3-2, the *in vitro* system used with human samples supports the process of extrapolation to humans.

Scheme 3-2



The overall approach described in this section for extrapolation to humans of the lead toxic effect observed in animals is summarised in Scheme 3-3. This lead effect is usually the most sensitive endpoint of those affected adversely by exposure to the test substance. Though an effect may be eliminated on the basis that its mode of action is non-relevant for humans, it cannot be concluded from this that no hazard exists for the substance under consideration. There are two likely scenarios: either a different endpoint should be selected, which will then become the new 'lead' effect to be examined as a basis for the assessment of human risk. Alternatively, the endpoint under consideration may still be used in the risk assessment but taking account of the factors that determine the species difference to establish a realistic margin of safety, e.g. based on more sophisticated dosimetry.

Scheme 3-3



[1] The Hill criteria may not be met if the postulated mode of action is incorrect or if the available evidence is inadequate to support the hypothesis. In such cases the default factors for species extrapolation should be applied. Alternatively, a decision could be made to develop and test an alternative hypothesis for the mode of action or to conduct additional studies towards meeting the Hill criteria.

4. THE APPROACH IN PRACTICE

The evaluation of the potential for a chemical to adversely affect human health begins with a review of the toxicological data set of which the major part generally comprises the results of experimental studies conducted according to regulatory guidelines. A structured approach for evaluating the outcome of toxicology studies to identify the substance-induced adverse effects is described in ECETOC Technical Report No 85 (ECETOC, 2002). From such studies, where more than one adverse effect arises from exposure to the substance under evaluation, the lead effect is identified and used as the basis for assessment of human risk.

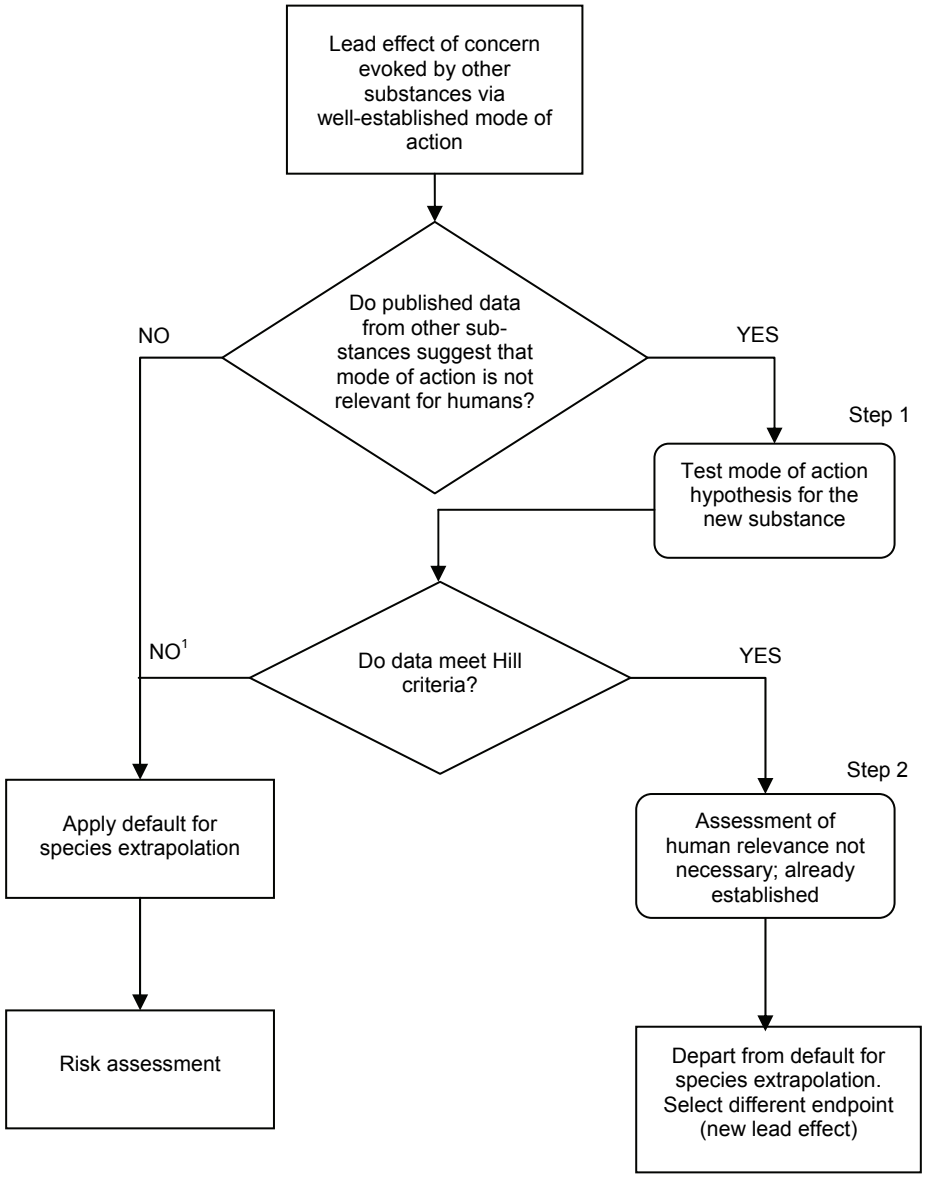
The default assumption made is that the effect is directly relevant to humans and safe levels of exposure are defined by application of assessment factors to the NOAEL established for that effect in the most sensitive species. Further details and guidance on the derivation and application of appropriate assessment factors in human health risk assessment can be found in ECETOC Technical Report No 86 (ECETOC, 2003).

However, where the data indicate that the effect is likely to be of questionable relevance to humans, the following structured process for confirming the decision to depart from the default approach should be followed.

4.1 An established mode of action

Where a 'new' substance under investigation appears to be exerting its lead effect via a mode of action that is similar to one that has been well-studied and established as one that is non-relevant to humans, it may be sufficient to confirm the non-relevance of the effect in the 'new' substance by examining its potential to evoke one or more of the key events. This approach, summarised in Scheme 4-1, is illustrated by the following examples in which the key events in the toxicological process of well-studied and accepted mechanisms are proposed as sufficient evidence for confirming that a 'new' substance would act via the same mechanism of toxicity.

Scheme 4-1

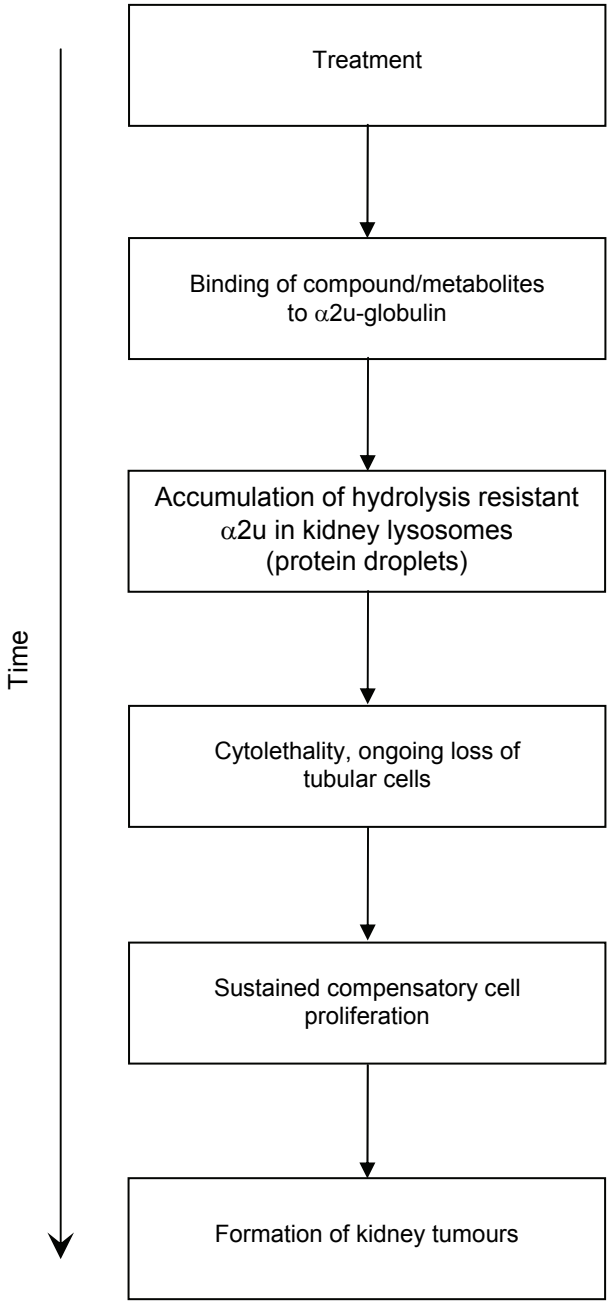


[1] The Hill criteria may not be met if the postulated mode of action is incorrect or if the available evidence is inadequate to support the hypothesis. In such cases the default factors for species extrapolation should be applied. Alternatively, a decision could be made to develop and test an alternative hypothesis for the mode of action or to conduct additional studies towards meeting the Hill criteria.

4.1.1 α2u-Globulin-mediated nephropathy in male rats

Where nephrotoxicity or increased kidney tumour rates occur exclusively in male rats (after subacute / subchronic or long-term treatment respectively), the suspicion should be that these changes are α2u-globulin-mediated. The sequence of critical events for this mode of action is presented in Scheme 4-2.

Scheme 4-2



α2u-Globulin-mediated nephropathy is a highly species- and sex-specific syndrome. No protein similar to α2u-globulin (α2u) has been detected in humans, indicating that humans are not at risk for kidney toxicity and tumour formation on exposure to substances that operate through a α2u mediated mode of action (Olson *et al*, 1990; Borghoff and Lagarde, 1993). However, the question arises as to which parts of this sequence of events have to be corroborated experimentally to confirm the hypothesis for a 'new' substance.

The US EPA (1991a) proposed minimal requirements that included in particular, male rat specific nephrotoxicity and carcinogenicity induced by a non-genotoxic substance. The other requirements included demonstration of an increase in the number and size of protein droplets, identification of α2u as the protein accumulating in the droplet and pathology characteristic of the accumulation of protein.

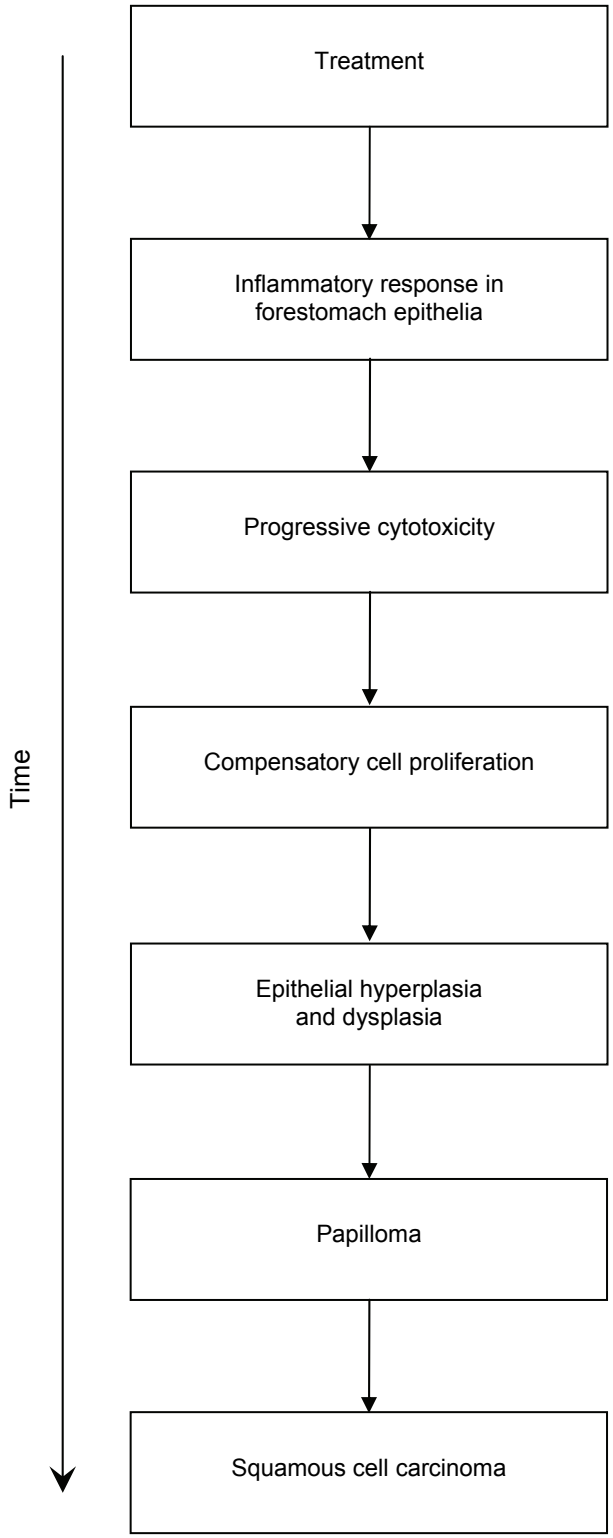
Studies of only a few days duration would be sufficient to demonstrate the accumulation of protein droplets in the renal tubular cells by immunohistochemistry using specific α2u antibodies. Accordingly, if under these conditions unequivocal evidence establishes that the accumulated protein droplets are essentially α2u, the Task Force believes that no further investigations are needed.

4.1.2 Forestomach tumours

Where long-term oral treatment of rats and/or mice with a non-genotoxic substance has revealed an increased incidence of forestomach tumours, this should immediately be considered rodent-specific, based on the knowledge that the forestomach is not present in humans, primates or carnivores, and the large amount of information on other substances that have induced tumours at this site in rodents. Further supportive evidence can be obtained by a careful analysis of tumour incidences at other locations (which in general should not be increased) as well as non-neoplastic effects in the oesophagus, forestomach and glandular stomach. In this case hyperplastic changes should not be present in the oesophagus and the glandular part of the stomach or those forestomach parts not affected by papillomas / carcinomas.

The sequence of events shown in Scheme 4-3 provides further guidance on confirming the hypothesis of a non-genotoxic, rodent-specific effect.

Scheme 4-3


Treatment
Inflammatory response in forestomach epithelia
Progressive cytotoxicity
Compensatory cell proliferation
Epithelial hyperplasia and dysplasia
Papilloma
Squamous cell carcinoma
Time

According to this scheme, it would be appropriate to conduct a short-term (7-28 days) study in the respective species with routine histology focused on the forestomach (especially limiting ridge), the oesophagus and the glandular stomach. This study could be restricted to the sex showing increased sensitivity to this effect.

If the results of this investigation match the overall picture as described above, no further special investigations should be necessary to confirm that the effect is the result of a mode of action that is not relevant to humans.

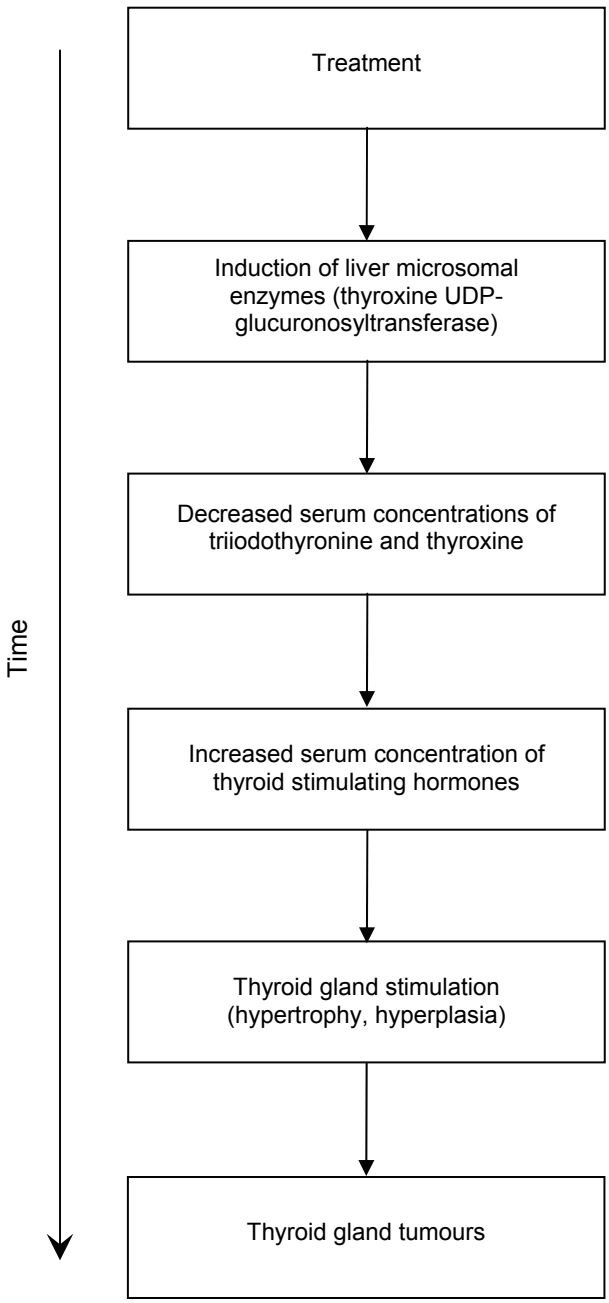
4.1.3 Thyroid gland stimulation by enzyme inducers

Various modes of action are known by which chronic exposure of rats to chemical substances results in the formation of thyroid gland tumours.

If one such substance of interest has been shown to be non-genotoxic, then one possible mode of action is through its ability to cause an imbalance in thyroid hormone metabolism. The sequence of events for this mode of action is shown in Scheme 4-4.

Among such non-genotoxic substances are liver enzyme inducers that increase the rate of the hepatic clearance of thyroid hormones. Decreased serum thyroid hormone, due to increased hepatic clearance, results in a compensatory increase in pituitary thyroid stimulating hormone (TSH) that can exert a tumour promoting effect. The ability of a substance to induce liver enzymes, specifically UDP-glucuronosyltransferase activity towards thyroxine (T4) can be measured using liver microsomes from treated rats. *In vivo* studies can be conducted at the dose and route of exposure that results in tumour induction, to demonstrate induction of liver enzymes coinciding with decreased triiodothyronine (T3) and T4 serum levels and a compensatory increase in the plasma level of TSH (McClain, 1992; McClain *et al*, 1989). These special investigations will confirm whether or not the substance under study exerts its thyrotoxic effect via this mode of action that is not relevant to humans.

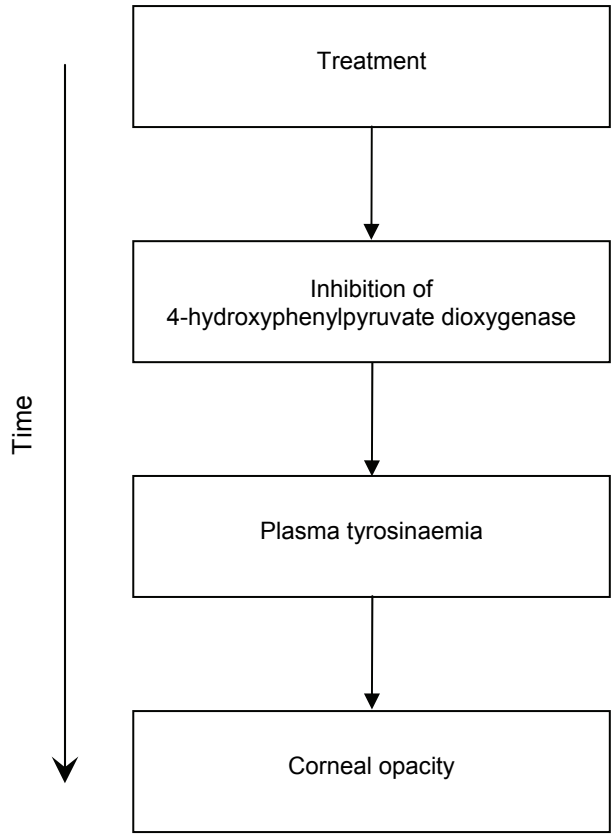
Scheme 4-4


Treatment
Induction of liver microsomal enzymes (thyroxine UDP-glucuronosyltransferase)
Decreased serum concentrations of triiodothyronine and thyroxine
Increased serum concentration of thyroid stimulating hormones
Thyroid gland stimulation (hypertrophy, hyperplasia)
Thyroid gland tumours
Time

4.1.4 Tyrosinaemia and corneal opacity in the rat

Reports of ocular opacity in rat studies might suggest that the lesions were caused by the presence of high concentrations of tyrosine, following inhibition of 4-hydroxyphenyl pyruvate dioxygenase (HPPD). This mechanism is displayed in Scheme 4-5. In mice and humans, even under conditions of strong HPPD inhibition, tyrosine concentrations will not increase to levels high enough to induce ocular toxicity and hence, this toxicity observed in the rat is inappropriate for extrapolation to humans.

Scheme 4-5



Ophthalmoscopy and histopathology will clearly be critical in determining whether the lesion in question shows the same features as that reported by Robinson (1995). Inflammation of the cornea (which is relatively rare), keratitis, life history and rapid regression of the lesion when treatment is removed, are the distinct features of this lesion.

Triketones induce ocular opacity in rats by causing a severe tyrosinaemia following a highly specific inhibition of the tyrosine catabolic enzyme, HPPD. If another or additional enzyme of the tyrosine catabolic cascade, such as tyrosine aminotransferase (TAT), were to be inhibited even partially by the substance of interest, the severe tyrosinaemia produced could also result in similar corneal lesions. It is important to realise that, if ocular opacity was reported for a substance that causes tyrosinaemia through partial inhibition of TAT, due to the different pathogenesis of the lesion the same biological effect of corneal opacity, could in those circumstances be relevant to humans. It is therefore critical, to demonstrate that HPPD is specifically inhibited (Provan *et al*, 1999).

Hence, in assessing the weight of evidence that would support the inclusion of a new substance into a generic class of HPPD inhibitors, the species / sex / age differences in response (severity of tyrosinaemia, incidence of corneal opacity) need to be consistent with that reported in the literature for substances where this mechanism is proven. In addition to proving that the corneal opacity is the same as that reported for model HPPD inhibitors, the fundamental cause of the tyrosinaemia must also be clearly elucidated and it should be known if the substance directly affects other enzymes of tyrosine catabolism.

4.1.5 Other modes of action whose human relevance has been questioned

Further examples of generic, non-genotoxic modes of action that may lead to effects in susceptible species but whose relevance to humans has been questioned are given in Table 4-1. Where such effects are observed for a ‘new’ chemical substance, investigation of the critical elements of the mode of action may lead to the conclusion that this aspect of toxicity is not relevant for humans and justify departure from the default approach for assessment of risk.

Table 4-1: Effects with modes of action specific to susceptible species

End point / Effect	Mode of Action	Species / Sex Sensitivity	Type of Difference	References
Ovarian tumours	Receptor mediated; induction of mesovarian leiomyomas in rats considered to be consequence of intense, and persistent, β-receptor stimulation	Rats + + Mice - Hamsters - Non-rodents - Humans - (No effects in clinical experiments)	Toxicodynamic (qualitative)	Nelson and Kelly, 1971; Jack *et al*, 1983; Kelly *et al*, 1993; Amemiya *et al*, 1984; Colbert *et al*, 1991; Poynter *et al*, 1978; Libretto, 1994
Mammary gland tumours	Acceleration of normal age-related perturbations of oestrus cycle; resulting in increased exposure to endogenous oestrogen and prolactin	Sprague Dawley Rats + + + Fischer 344 Rats - Mice + - Hamsters - Humans - (Different pattern of reproductive senescence between women and female S-D rats)	Toxicodynamic (qualitative and quantitative)	Chapin *et al*, 1996; Simpkins *et al*, 1998; Eldridge *et al*, 1998; Eldridge *et al*, 1994, Wetzel *et al*, 1994; Connor *et al*, 1998; O'Connor *et al*, 2000; Thakur *et al*, 1998
Bladder tumours	Formation of urinary calcium phosphate-containing precipitate or cytotoxicity with subsequent cell proliferation leading to urothelial bladder tumours	Rats + + Mice - Hamster - Humans -	Toxicokinetic (qualitative)	Capen *et al*, 1999; Cohen, 1983, 1995, 1999; Cohen *et al*, 1990; Squire, 1985; Fukushima *et al*, 1983
Leydig cell tumours	Disruption of hypothalamic-pituitary-testicular axis; resulting in sustained increase in circulatory luteinising hormone and increased incidence of Leydig cell tumours	Rats + + Mice + - Humans -	Toxicodynamic (quantitative)	Clegg *et al*, 1997; Christensen and Peakock, 1980; Cook *et al*, 1992, 1997, 1999

Table 4-1: Effects with modes of action specific to susceptible species *(cont'd)*

End point / Effect	Mode of Action	Species / Sex Sensitivity	Type of Difference	References
Liver tumours	Induction of hepatic peroxisome proliferation with hepatic cell proliferation; resulting in preneoplastic lesions and eventual tumour formation	Rats + + Mice + + Guinea pigs - Cats - Dogs - Primates - Humans -	Toxicodynamic (qualitative)	Foxworthy *et al*, 1990; Ashby *et al*, 1994; IARC, 1995b, Lake, 1995a, b; Cattley *et al*, 1998; Wada *et al*, 1992; Biegel *et al*, 1992; ECETOC, 1992

4.2 An observed effect with an unknown mode of action

Where a 'new' substance under investigation appears to be exerting its lead effect via a mode of action that has not been already described and established as generic for other substances, the default assumption of relevance to humans must be applied unless the data suggest otherwise. In such cases a thorough application of the approach outlined in Section 3 is recommended in order to justify departure from the default extrapolation process. As shown in Scheme 3-3, having identified the lead effect and found evidence to doubt its relevance to humans, e.g. it appears to be species-specific; the first major step to be taken is the elucidation of the mode of action. Once this has been established, the second major step is to evaluate the relevance of this mode of action to humans.

As already emphasised, it is a challenging task to identify and demonstrate conclusively a sequence of key events for a new type of effect, and to establish within this sequence at least one event that will not take place in humans. In order to achieve this goal convincingly, in most cases, extensive additional experimentation will be unavoidable.

Coumarin, methylene chloride and formaldehyde are examples of substances which have undergone extensive investigation to elucidate their mechanism and mode of action to demonstrate non-relevance of the findings to humans; their case summaries are given in Appendix A.

5. CONCLUSION

The assessment of hazard and risk to humans that might arise from exposure to a chemical substance relies predominantly on the extrapolation of data generated in studies with experimental animals. The objective of such risk assessments should be to describe, with as little uncertainty as possible, the potential for unwanted health effects arising in humans due to their exposure to potentially hazardous chemicals. In practical terms, the assumption is made that the adverse effects evoked by the substance in various regulatory tests would also be expressed in humans and the risk assessment based on the most critical or 'lead' effect in the most sensitive animal species. To account for uncertainties including the possibility of greater sensitivity of humans to the effects of the chemical, assessment factors are applied in deriving 'safe' exposures for humans.

For a significant number of substances, it has been shown that adverse effects observed in animal studies would not be manifest in humans and would therefore not be a relevant basis for hazard or risk assessment. It is generally accepted that the expression of toxicity in a mammalian system is dependent on a sequence of key events taking place, each of which is critical for the ultimate effect to be observed. Thus, for a specific toxic endpoint, to justify deviating from the default approach in risk assessment, it should be sufficient to identify the key events in the process (mode of action) and establish that at least one of them would not occur in humans, or would occur only to a much lesser extent. From such knowledge it could be concluded that the toxic outcome would not be observed in humans, or be observed only at much higher and possibly irrelevant exposure levels.

A number of these cases are described in this report and used to develop a structured approach to justify departure from the use of default assessment factors. In evaluating a 'new' chemical substance, having identified the lead effect from the toxicology data set, before proceeding with the default approach, the experimental animal data should be examined to establish whether:

a) the effect of the chemical to be assessed differs qualitatively or quantitatively in different test species;
b) the effect of the chemical to be assessed is generically similar to other compounds with a well-established mode of action that is not considered relevant to humans.

In the case of either or both, it would be appropriate to proceed by analysing the data by applying the two-step approach detailed in Chapters 3 and 4, namely:

1. to identify the mode of action of toxicity in the susceptible species;
2. to assess the relevance of the identified mode of action for humans.

Where this analysis confirms a qualitative difference in the sensitivity between the experimental animals and humans to the toxic effect, this endpoint should not be used in assessing human risk. The toxicity database should be examined to identify the next most sensitive endpoint or lead effect (relevant to humans) to be used as a basis for the assessment. Chronic nephropathy and subsequent kidney tumour formation in the male rat via α2u and forestomach tumour formation in rodents are examples where a qualitative difference has been demonstrated convincingly.

Where it is shown that the difference between animals and humans in expression of the adverse effect is quantitative, the evaluation of human hazard and risk must be undertaken using a case by case approach. This is because the outcome will depend on the extent of the difference, i.e. it will determine whether it would be appropriate to select a different sensitive endpoint or whether it would justify the derivation of more appropriate risk estimates for humans in place of applying the default assessment factors. An example of such is formaldehyde, where the risk assessment for respiratory tract effects was based on use of biomarkers in the target tissues to establish a realistic margin of safety.

Where the evidence supports the case for species differences in sensitivity, qualitative or quantitative, which have not been investigated or described for other substances, the process for establishing the non-relevance to humans of that effect is likely to require extensive additional investigations.

The use of this approach to identify those substances, which express toxicity in experimental animals via a mode of action that is not relevant to humans, will enable their more realistic risk assessment. Furthermore, adopting a 'mode of action' approach should considerably speed up the risk assessment process and avoid unnecessary animal experimentation.

ABBREVIATIONS

2,4-D	2,4-Dichlorophenoxyacetic acid
2,4,5-T	2,4,5-Trichlorophenoxyacetic acid
α-KG	α-Ketoglutarate
α2u	α2u-Globulin
α2u-N	α2u-Globulin nephropathy
ADME	Absorption, distribution, metabolism and excretion
AIHC	American Industry Health Council
AUC	Area under curve
CIIT	Formerly: Chemical Industry Institute of Toxicology Now: Centers for Health Research
DNA	Deoxyribonucleic acid
EC	European Community
EU	European Union
HPPD	4-Hydroxyphenyl pyruvate dioxygenase
IARC	International Agency for Research on Cancer
JMAFF	The Japanese Ministry of Agriculture, Forestry and Fisheries
MCPA	4-Chloro-2-methylphenoxyacetic acid
NOAEL	No observed adverse effect level
NTBC	2-(2-Nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione
T3	Triiodothyronine
T4	Thyroxine
TAT	Tyrosine aminotransferase
TRH	Thyrotropin-releasing hormone
TSH	Thyroid stimulating hormone
UDP	Uridine diphosphate
US EPA	US Environmental Protection Agency
WHO	World Health Organization

APPENDIX A: TOXICOKINETIC DIFFERENCES BETWEEN ANIMALS AND HUMANS

The following examples are well-documented in the literature, but for ease of reference details are provided below.

Coumarin

Coumarin is used clinically in the treatment of high-protein oedema and has been tested in several clinical trials for the treatment of certain malignancies. Due to its use as a drug, information is available concerning its toxicity and metabolism in humans.

The liver is the major target organ for toxicity and carcinogenicity of coumarin in rats (National Toxicology Program, 1993). At levels that exceeded the maximum tolerated dose (5000 ppm), chronic dietary administration of coumarin resulted in increased incidences of cholangiocarcinomas and parenchymal liver cell tumours (Carlton *et al*, 1996). Other species, such as the Syrian hamster and the baboon, are much more resistant to coumarin-induced toxicity (Evans *et al*, 1979). In the Syrian hamster, subchronic and/or chronic administration failed to induce vacuolar degeneration, apoptosis, bile duct degeneration or sustained stimulation of DNA synthesis (Lake *et al,* 1990; Lake and Grasso, 1996; Ueno and Hirono, 1981). No increased incidence of liver tumours was observed in the chronic studies. Baboons fed at the maximum tolerated dose for up to 2 years showed no evidence of biliary hyperplasia or fibrosis, and lesions were limited to some ultrastructural changes compatible with early cell damage (Evans *et al*, 1979).

In rats, the main route of coumarin metabolism is via oxidation to coumarin 3,4-epoxide and only minor amounts of 7-hydroxylation metabolites are formed and excreted (Lake, 1999). Coumarin 3,4-epoxide is rapidly metabolised to o-hydroxyphenylacetaldehyde (Ratanasavanh *et al*, 1996; Born *et al*, 1997); and this metabolite, together with the epoxide, is probably responsible for the toxicity and carcinogenicity in this species. *In vitro* studies have confirmed that the formation of o-hydroxyphenylacetaldehyde is the predominant pathway in Fischer 344 rats (Born *et al*, 2000). In contrast to coumarin, dihydrocoumarin (which lacks the 3,4-double bond), as well as some coumarin metabolites (such as 3- and 7-hydoxycoumarin), produce little toxicity *in vivo* or *in vitro* in rat hepatocytes (Lake *et al*, 1989).

In other species such as the gerbil (Lake *et al*, 1992), Syrian hamster (Lake *et al*, 1990) or baboon (Evans *et al*, 1979), the 7-hydroxylation pathway is predominant, although the ratio between this pathway and 3,4-epoxidation may depend on the dose administered (Born *et al*, 2000).

In humans, coumarin metabolism occurs predominantly via 7-hydroxylation (Egan *et al*, 1990). Studies with microsomes from 12 different human subjects demonstrated that, even in clinical trials where large dosages of coumarin are administered, epoxidation contributes little to the elimination of coumarin (Born *et al*, 2000). Little evidence of hepatotoxicity was reported in humans given coumarin in clinical trials to treat various malignancies and chronic infections (Cox *et al*, 1989; Dexeus *et al*, 1990).

The available experimental evidence suggests that the predominance of the 7-hydroxylation detoxification pathway significantly contributes to the absence of hepatotoxicity and liver carcinogenicity in the Syrian hamster and in primates including humans (Lake, 1999). The hepatotoxicity and liver carcinogenicity findings in the rat are most likely related to the formation of a reactive epoxide metabolite and o-hydroxyphenylacetaldehyde.

The data available for coumarin suggest that the hepatotoxicity and liver carcinogenicity in rats is inappropriate for extrapolation to humans.

Methylene chloride

Liver and lung tumours were observed exclusively in mice on long-term exposure to methylene chloride (Mennear *et al*, 1988). The carcinogenic mode of action comprises its metabolism to a reactive glutathione conjugate, followed by covalent binding of this conjugate to DNA, leading ultimately to cancer (Green, 1997). DNA-protein cross-linking as a consequence of formaldehyde formation from the reactive glutathione metabolite may also contribute to tumour formation. In the lung, cytotoxicity (to Clara cells) and increased cell proliferation were probable contributory factors (Burek *et al*, 1984). The species specificity of tumour formation is a consequence of the presence of a theta-glutathione S-transferase with a high activity and cellular as well as nuclear localisation, which seems to be unique to the mouse. In other species including the rat, hamster and humans, the glutathione conjugate pathway is much less important (Green, 1997). Although the high nuclear enzyme activity in the mouse resulted in DNA damage in liver and lung cells, DNA damage could not be detected *in vitro* in hepatocytes from hamsters and human. In rat hepatocytes, DNA damage was only seen at high concentrations of methylene chloride that could not be achieved *in vivo*, thus explaining the lack of carcinogenicity in the rat (Graves *et al*, 1995).

The data available for methylene chloride suggest that the carcinogenic response in the mouse is unique for this species and inappropriate for extrapolation to humans.

Formaldehyde

Inhalation bioassays with formaldehyde revealed nasal squamous cell carcinoma in rats and mice (Kerns *et al*, 1983; Albert *et al*, 1982; Feron *et al*, 1988; Woutersen *et al*, 1989). Formaldehyde causes mutations and DNA damage in bacteria. It induces gene mutations, chromosomal aberrations, sister chromatid exchanges, DNA strand breaks, and DNA-protein cross-links in mammalian cells (IARC, 1995a). The induction of mutations by formaldehyde is dependent on the rate of cell replication. High rates of cell replication are a normal tissue response to cytolethality and are induced in the rat nasal epithelium by high concentrations of formaldehyde (Monticello *et al*, 1996). Tumour formation is only observed at dose levels that cause tissue damage and regenerative cell proliferation. DNA-protein cross-links are formed when inhaled formaldehyde reaches the nuclei of nasal epithelial cells (Casanova *et al*, 1994). Enhanced cell replication in the presence of DNA-protein cross-links is proposed to be the mode of action. The rate of protein-DNA cross-link formation is directly related to the concentration of free formaldehyde in nasal epithelial cell nuclei. Thus, DNA-protein cross-links provide a biomarker for effective target tissue dose of formaldehyde (Casanova *et al*, 1989; Casanova *et al*, 1991; Casanova *et al,* 1994; Heck *et al*, 1990). DNA-protein cross-link measurements in rats and monkeys showed steep exposure-biomarker relationships in both species (Casanova-Schmitz *et al*, 1984; Casanova *et al*, 1989; Casanova *et al*, 1991; Casanova *et al,* 1994). Physiologically based kinetic modelling using rat data and incorporating species differences in physiological parameters allowed the correct prediction of DNA-protein cross-link values for monkeys (Casanova *et al*, 1991; Casanova *et al,* 1994). Because of similarity of the monkey nasal anatomy and air flow characteristics to the human, it is assumed to be the more appropriate species for estimating human risk.

The formaldehyde example demonstrates the usefulness of dose-response information for a key precursor event for risk assessment (Haber *et al*, 2001). DNA-protein cross-links as a target tissue dosimeter in place of inhaled formaldehyde concentration allowed more sophisticated risk assessment which resulted in lower risk estimates for humans (US EPA, 1991b; California Air Resources Board, 1992; CIIT, 1999).

Phenoxyacetic acids

A number of experimental studies have demonstrated that the dog is particularly susceptible to the adverse effects of the weak organic acids 2,4-dichlorophenoxyacetic acid (2,4-D), 4-chloro-2-methylphenoxyacetic acid (MCPA) and the related herbicides triclopyr and 3,5,6-trichloro-2-pyrinyloxyacetic acid. (Arnold and Beasley, 1989; Gehring and Betso, 1978). As a class, these compounds are known to have similar pharmacokinetic properties.

The kinetics and metabolism of MCPA were investigated in rats and dogs after oral dosing with (14C)-MCPA at 5 mg kg-1 (Lappin *et al*, 2002). Radioactivity was eliminated from plasma significantly more quickly in the rat than in the dog, resulting in a more than 10-fold higher AUC (area under the curve) value (based on total radioactivity) in dog compared with rat.

The toxicological profile of 2,4-D is much like that of MCPA. A toxicokinetic study designed to investigate whether similar conclusions could be drawn for 2,4-D (van Ravenzwaay *et al*, 2003) showed that elimination of the radioactive dose of 2,4-D from rat plasma was significantly more rapid than in the dog. The approximate half-life values were 1.3-3.4 h for the rat and 99-134 h for the dog following a 5 or 50 mg kg-1 dose, respectively. Comparison of the plasma AUC showed that the values for dog were 27 (high dose) to 232 (low dose) times higher than the corresponding values in the rat. These results showing higher systemic exposure to the test material in the dog are consistent with the increased sensitivity of this species to 2,4-D toxicity.

Similar compounds such as dichlorprop and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) are known to be excreted by active renal mechanisms (Beitz *et al*, 1985). Basolateral accumulation of 2,4-D has been shown to be mediated by 2,4-D/α-ketoglutarate (α-KG) exchange, coupled indirectly to Na+/α-KG co-transport in the same way as ρ-aminohippurate and fluorescein (Villalobos *et al*, 1996). Moreover, in *in vitro* studies with kidney slices, 2,4-D was shown to concentrate in the kidney tissue through an active transport mechanism (Berndt and Koschier, 1973).

Investigations by Timchalk and Nolan, 1997; van Ravenzwaay et al, 2003; and Lappin *et al*, 2002 show clearly that dogs, in contrast to rats, are deficient in their ability to excrete a range of organic acids. At least for 2,4-D, metabolism is required in the dog before the compound can be excreted (van Ravenzwaay *et al*, 2003). In rats, monkeys or in humans, metabolism does not seem to be a prerequisite for excretion (Timchalk and Nolan, 1997).

Comparative pharmacokinetics of phenoxyacetic acids and structurally related organic acids in a variety of species including humans were evaluated using allometric parameter scaling (Timchalk, 2004). For both 2,4-D and MCPA, the dog plasma half-life and renal clearance rates could not be reasonably scaled across species. For all other species evaluated, including humans, scaling of the same pharmacokinetic parameters was possible. These findings demonstrate that the dog is not an appropriate model to investigate the toxicity of phenoxyacetic and other structurally related organic acids for humans. The European Union also concluded that for 2,4-D, the dog was not the relevant species for setting of regulatory values for human health risks and that these should be based on a NOAEL from rodent studies (EU, 2001).

APPENDIX B: TOXICODYNAMIC DIFFERENCES BETWEEN ANIMALS AND HUMANS

α2u-Globulin-mediated kidney tumours in male rats

Chemically-induced α2u-globulin nephropathy (α2u-N) occurs in male rats following exposure to a variety of non-genotoxic substances (Borghoff *et al,* 1990; Hard *et al,* 1993; Swenberg and Lehman-McKeeman, 1999). Chronic exposure of male rats to such substances induces a low incidence of renal tumours in male rats. Following exposure in the male rat, the specific low molecular weight protein α2u-globulin (α2u) accumulates in the form of protein droplets in proximal tubule epithelial cells. Normally, α2u is synthesised in the male rat liver, secreted into blood and filtered at the glomerulus, with approximately 50-60% reabsorbed into kidney proximal tubular cells under physiological conditions (Roy and Neuhaus, 1966; Neuhaus, 1986; MacInnes *et al*, 1986). Substances that cause α2u-N bind reversibly to α2u (Swenberg and Lehman-McKeeman, 1999; Prescott-Matthews *et al*, 1999) and, in addition, increase the rate of direct uptake of α2u into lysosomes of proximal tubular cells (Cuervo *et al*, 1999). The chemical-α2u complex is more resistant to hydrolytic degradation that leads to its accumulation as protein droplets (Lehman-McKeeman *et al*, 1990). Cytolethality occurs when this accumulation reaches a certain level. As a result of cell death and degeneration, there is compensatory cell proliferation in the region where the α2u accumulates (Short *et al*, 1987). This increase in cell proliferation enhances the likelihood of spontaneous mutational events and may result in the clonal expansion of initiated cells leading to tumour formation (Butterworth *et al*, 1992).

α2u-N is a highly species- and sex-specific syndrome. No protein similar to α2u has been detected in humans, indicating that humans are not at risk for kidney toxicity and tumour formation on exposure to substances that operate through an α2u mediated mode of action (Olson *et al*, 1990; Borghoff and Lagarde, 1993).

Forestomach tumours

In rodents the stratified squamous epithelium of the oesophagus continues into the cardiac glandular portion of the stomach to form the covering of the forestomach. These stomach structures are not present in humans, monkeys, dogs, or guinea pigs (Grice, 1988). The rodent forestomach is the target organ for a large number of non-genotoxic as well as genotoxic substances (Kroes and Wester, 1986). Exposure to relatively high oral doses of certain non-genotoxic substances triggers a typical sequence of morphological changes. Initially, cellular proliferation leads to hyperplasia and, if exposure is continued, to papillomas and finally to squamous cell carcinomas (Clayson *et al*, 1991; Iverson, 1995). If treatment is stopped at a phase

where tumours have not yet developed, these changes are fully reversible (Altmann *et al*, 1985; Hirose *et al*, 1990; Kagawa *et al*, 1993). The oesophagus and the glandular part of the stomach are not usually affected (Ito *et al*, 1986; Wester and Kroes, 1988). Stimulation of cell division resulting in cell proliferation in the forestomach epithelium occurs within only a few days if high dosages are administered (Clayson *et al*, 1991; Iverson, 1995; Lutz *et al*, 1997; Verhagen *et al*, 1988). Cell proliferation is greatest in the squamous epithelium immediately adjacent to the glandular mucosa (i.e. the limiting ridge), and this is also the site at which most tumours develop (Powell and Berry, 1999). The link between cellular proliferation and the development of forestomach tumours has not yet been fully elucidated; nevertheless sustained stimulation of cell proliferation can lead to tumour formation (Clayson *et al*, 1991). There is a clear-cut threshold; dose levels which do not cause morphological changes (such as cell proliferation or hyperplasia) are not tumorigenic.

In species, including humans, that lack a forestomach comparable to rodents, the sequence of events described above cannot take place (Grice, 1988; Moch, 1988). Moreover, the oesophagus (which cytologically resembles the forestomach epithelium), is not a target tissue either in rodents or non-rodents for tumour formation from such substances (Kroes and Wester, 1986). Consequently, there is no tumorigenic / carcinogenic potential for humans.

Thyroid gland stimulation by enzyme inducers

Thyroid cancer incidence in humans is low, while thyroid neoplasia is common in rodent, especially rat carcinogenicity studies. This is explained by two major differences between rodent and human thyroid physiology: (1) absence of a high affinity thyroid hormone-binding protein in rodents, with the consequence of much shorter plasma half-life of thyroid hormones in these species and (2) sustained stimulation of the thyroid gland by increased plasma levels of thyroid stimulating hormone (TSH) is associated with thyroid follicular cell neoplasia in rodents but not in humans (Curran and DeGroot, 1991).

Homeostasis of thyroid hormone synthesis and secretion is normally controlled by a sensitive feedback mechanism that involves the hypothalamus, the pituitary and the thyroid gland (Capen *et al*, 1991; Paynter *et al*, 1988, Owen *et al*, 1973; Atterwill and Brown, 1988). Of particular importance in this feedback mechanism is TSH, which is secreted by the pituitary and stimulates the thyroid gland to synthesise and release thyroid hormones. The rate of TSH release is controlled by the amount of thyrotropin-releasing hormone (TRH) released by the hypothalamus as well as by circulating triiodothyronine (T3) and thyroxine (T4) levels. When circulating T3 and T4 levels are reduced, the pituitary is triggered to secrete TSH, which in turn stimulates the thyroid to produce T4 and T3. The thyroid produces predominantly T4 which is either 5'-deiodinated to give the more active hormone T3 or undergoes inner ring deiodination to form rT3

which has no known hormonal activity. Apart from T4 deiodination, degradation of thyroid hormones occurs mainly in the liver where both T4 and T3 are conjugated to the respective glucuronide or sulphate that are then excreted via bile into the intestine (Hill *et al*, 1989; van Raaij *et al*, 1993).

Substances that induce xenobiotic hepatic metabolising enzymes can increase the plasma clearance of thyroid hormones; for example, increased hepatic glucuronidation rate of T4 as a consequence of thyroxine UDP-glucuronosyltransferase induction (Capen *et al*, 1991; McClain *et al*, 1989; McClain, 1992). The absence of specific plasma binding proteins for thyroid hormones renders rodents more susceptible to increased rates of thyroid hormone removal from blood. As a feedback response, increased plasma TSH levels will stimulate the gland, accelerate thyroid hormone production, and increase DNA synthesis as well as cell proliferation (Hill *et al*, 1989; Capen *et al*, 1991). On chronic exposure, nodular hyperplasia and finally follicular adenomas and carcinomas are formed (McClain *et al*, 1989).

Despite a common physiology between rodents and humans, humans are much more resistant to chemical disturbances of the thyroid-pituitary axis and do not develop cancer from its chemically-induced perturbation (McConnell, 1992; Capen *et al*, 1991; Capen, 1992; McClain, 1992).

Triketone-mediated tyrosinaemia and corneal opacity

Various triketones, including 2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione (NTBC) and mesotrione, have been reported to cause corneal opacity in rats but not in mice (Lock *et al*, 1996, 2000). Investigative toxicology studies have revealed that this species-specific ocular toxicity is caused by the action of high concentrations of tyrosine at the target site, rather than by the direct action of xenobiotics, or metabolites thereof, on the eye (Robinson 1995; Lock *et al*, 1996; Ellis *et al*, 1995).

The basis for the species difference in expression of toxicity is due to differences in the disposition of tyrosine, the ultimate toxiphore, between rats and mice (Provan *et al*, 1999). The rate-limiting step in the removal of excess tyrosine is its conversion to 4-hydroxyphenylpyruvate by tyrosine aminotransferase (TAT) (Goldsmith, 1983). Rats have much lower activity of hepatic TAT than mice and are therefore unable to prevent the build up of high and toxic tyrosine levels in plasma (Provan *et al*, 1999). In contrast, mouse hepatic TAT activity is of sufficient magnitude to prevent tyrosine concentrations of reaching toxic levels.

Data indicate the activity of hepatic TAT in humans to be similar to mice (Henderson *et al*, 1981), suggesting that the mouse is a more appropriate model than the rat to investigate the potential hazard of mesotrione-induced ocular toxicity in humans.

NTBC is approximately 1000-fold more potent than mesotrione in its inhibition of 4-hydroxyphenyl pyruvate dioxygenase (HPPD) (Hall *et al*, 2001). For NTBC, which is used internationally in the treatment of children suffering from the rare hereditary disease Tyrosinaemia Type I, there is currently over 1000 patient-years clinical experience without evidence for adverse ocular effects (personal communication with Swedish Orphan AB). Furthermore studies have been conducted with NTBC and mesotrione in healthy male volunteers (Hall *et al*, 2001). With mesotrione, only marginally increased plasma tyrosine concentrations were observed which quickly returned to control levels. Steady state plasma tyrosine concentrations with NTBC were also much lower than those observed in the rat. No ocular toxicity was associated on human exposure to either NTBC or mesotrione.

The corn herbicide 2-(4-methylsulfonyl-2-nitrobenzoyl-1,3-cyclohexanedione (mesotrione) and other triketones, such as NTBC are inhibitors of HPPD (Ellis *et al*, 1995; Provan *et al*, 1999). In mammals, HPPD is the second enzyme of the catabolic cascade for removal of excess dietary tyrosine. The biological consequence of HPPD inhibition in mammals has been shown to be a rise in plasma tyrosine.

In mice and humans, even under conditions of strong HPPD inhibition, tyrosine concentrations will not increase to levels high enough to induce ocular toxicity and hence, this toxicity observed in the rat is inappropriate for extrapolation to humans.

BIBLIOGRAPHY

Albert RE, Sellakumar AR, Laskin S, Kuschner M, Nelson N, Snyder CA. 1982. Gaseous formaldehyde and hydrogen chloride induction of nasal cancer in the rat. *J Natl Cancer Inst* 68(4):597-603.

Altmann HJ, Wester PW, Matthiaschk G, Grunow W, van der Heijden CA. 1985. Induction of early lesions in the forestomach of rats by 3-tert-butyl-4-hydroxyanisole (BHA). *Food Chem Toxicol* 23(8):723-731.

Amemiya K, Kudoh M, Suzuki H, Saga K, Hosaka K. 1984. Toxicology of mabuterol. *Arzneimittelforschung (Drug Res)* 34(11A):1680-1684.

Arnold EK, Beasley VR. 1989. The pharmacokinetics of chlorinated phenoxy acid herbicides: a literature review. *Vet Hum Toxicol* 31(2):121-125.

Ashby J, Brady A, Elcombe CR, Elliott BM, Ishmael J, Odum J, Tugwood JD, Kettle S, Purchase IF. 1994. Mechanistically-based human hazard assessment of peroxisome proliferator-induced hepatocarcinogenesis. *Hum Exp Toxicol* 13(Suppl2):1-117.

Atterwill CK, Brown CG. 1988. Mechanistic studies on the thyroid toxicity induced by certain drugs. *Arch Toxicol Suppl* 12:71-79.

Beitz H, Banasiak U, Gericke S, Clausing P. 1985. Toxicokinetics of herbicide dichlorprop and its leucinate and their action on liver mixed function oxidase in rats. *Arch Toxicol Suppl* 8:259-263.

Berndt WO, Koschier F. 1973. *In vitro* uptake of 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) by renal cortical tissue of rabbits and rats. *Toxicol Appl Pharmacol* 26(4):559-570.

Biegel LB, Hurtt ME, Frame SR, Applegate M, O'Connor JC, Cook JC. 1992. Comparison of the effects of Wyeth-14,643 in Crl:CD BR and Fisher-344 rats. *Toxicol SC* 19(4):590-597.

Borghoff SJ, Lagarde WH. 1993. Assessment of binding of 2,2,4-trimethyl-2-pentanol to low-molecular-weight proteins isolated from kidneys of male rats and humans. *Toxicol Appl Pharmacol* 119: 228-235.

Borghoff SJ, Short BG, Swenberg JA. 1990. Biochemical mechanisms and pathobiology of α2u-globulin nephropathy. *Annual Rev Pharmacol Toxicol* 30:349-367.

Born SL, Caudill D, Smith BJ, Lehman-McKeeman LD. 2000. *In vitro* kinetics of coumarin 3,4-epoxidation: Application to species differences in toxicity and carcinogenicity. *Toxicol Sci* 58:23-31.

Born SL, Rodriguez PA, Eddy CL, Lehman-McKeeman LD. 1997. Synthesis and reactivity of coumarin 3,4-epoxide. *Drug Metab Dispos* 25(11):1318-1324.

Burek JD, Nitschke KD, Bell TJ, Wackerle DL, Childs RC, Beyer JE, Dittenber DA, Rampy LW, McKenna MJ. 1984. Methylene chloride: a two-year inhalation toxicity and oncogenicity study in rats and hamsters. *Toxicol Sci* 4(1):30-47.

Butterworth BE, Conolly RB, Morgan KT. 1995. A strategy for establishing mode of action of chemical carcinogens as a guide for approaches to risk assessments. *Cancr Lett* 93(1):129-146.

Butterworth BE, Popp JA, Conolly RB, Goldsworthy TL. 1992. Chemically induced cell proliferation in carcinogenesis. International Agency for Research on Cancer, Lyon, France. *IARC Sci Publ* 116:279-305.

California Air Resources Board. 1992. Cancer risk assessment for airborne formaldehyde. Office of Environmental Health Hazard Assessment, Air Toxicology and Epidemiology Section.

Capen CC. 1992. Pathophysiology of chemical injury of the thyroid gland. *Toxicol Lett* 64-65:381-388.

Capen CC, DeLellis RA, Yarrington JT. 1991. Endocrine System. In Haschek WM and Rousseaux CG, eds, *Handbook of Toxicologic Pathology,* Academic Press, New York, 711-736.

Capen CC, Dybing E, Rice JM, Wilbourn JD. 1999. Species differences in thyroid, kidney and urinary bladder carcinogenesis. International Agency for Research on Cancer, Lyons, France. *IARC Sci Publ* 147.

Carlton BD, Aubrun JC, Simon GS. 1996. Effects of coumarin following perinatal and chronic exposure in Sprague-Dawley rats and CD-1 mice. *Toxicol Sci* 30:145-151.

Casanova M, Deyo DF, Heck HD. 1989. Covalent binding of inhaled formaldehyde to DNA in the nasal mucosa of Fischer 344 rats: Analysis of formaldehyde and DNA by high-performance liquid chromatography and provisional pharmacokinetic interpretation. *Toxicol Sci* 12(3):397-417.

Casanova M, Morgan KT, Gross EA, Moss OR, Heck HD. 1994. DNA-protein cross-links and cell replication at specific sites in the nose of F344 rats exposed subchronically to formaldehyde. *Toxicol Sci* 23(4):525-536.

Casanova M, Morgan KT, Steinhagen WH, Everitt JI, Popp JA, Heck HD. 1991. Covalent binding of inhaled formaldehyde to DNA in the respiratory tract of rhesus monkeys: Pharmacokinetics, rat-to-monkey interspecies scaling, and extrapolation to man. *Toxicol Sci* 17(2):409-428.

Casanova-Schmitz M, Starr TB, Heck HD. 1984. Differentiation between metabolic incorporation and covalent binding in the labelling of macromolecules in the rat nasal mucosa and bone marrow by inhaled [^{14}C]- and [^{3}H]formaldehyde. *Toxicol Appl Pharmacol* 76: 26-44.

Cattley RC, DeLuca J, Elcombe C, Fenner-Crisp P, Lane BG, Marsman DS, Pastoor TA, Popp JA, Robinson DE, Schwetz B, Tugwood J, Wahli W. 1998. Do peroxisome proliferating compounds pose a hepatocarcinogenic hazard to humans? *Regul Toxicol Pharmacol* 27(1Pt1):47-60.

Chapin RE, Stevens JT, Hughes CL, Kelce WR, Hess RA, Daston GP. 1996. Symposium overview: Endocrine modulation of reproduction. *Fundam Appl Toxicol*, 29(1):1-17.

Christensen AK, Peacock KC. 1980. Increase in Leydig cell number in testes of adult rats treated chronically with an excess of human chorionic gonadotropin. *Biol Reprod* 22(2):383-391.

CIIT. Formerly: Chemical Industry Institute of Toxicology; now: Centers for Health Research. 1999. Formaldehyde: hazard characterization and dose-response assessment for carcinogenicity by the route of inhalation. (Revised Edition). Chemical Industry Institute of Toxicology, Research Triangle Park, NC, USA. www.ciit.org.

Clayson DB, Iverson F, Nera EA, Lok E. 1991. Early indicators of potential neoplasia produced in the rat forestomach by non-genotoxic agents: the importance of induced cellular proliferation. *Mutat Res* 248(2):321-331.

Clegg ED, Cook JC, Chapin RE, Foster PMD, Daston GP. 1997. Leydig cell hyperplasia and adenoma formation: mechanisms and relevance to humans. *Reprod Toxicol* 11(1):107-121.

Cohen SM. 1983. Promotion in urinary bladder carcinogenesis. *Environ Health Perspect* 50:51-59.

Cohen SM. 1995. Role of urinary physiology and chemistry in bladder carcinogenesis. *Food Chem Toxicol* 33(9):715-730.

Cohen SM. 1999. Calcium phosphate-containing urinary precipitate in rat urinary bladder carcinogenesis. In: Capen C, Dybing E, Rice J, Wilbourn J, eds, *Species differences in thyroid, kidney and urinary bladder carcinogenesis.* International Agency for Research on Cancer, Lyons, France. *IARC Sci Publ* 147:175-189.

Cohen SM, Fisher MJ, Sakata T, Cano M, Schoenig GP, Chappel CI, Garland EM. 1990. Comparative analysis of the proliferative response of the rat urinary bladder to sodium saccharin by light and scanning electron microscopy and autoradiography. *Scanning Microsc* 4(1):135-142.

Colbert WE, Wilson BF, Williams PD, Williams GD. 1991. Relationship between *in vitro* relaxation of the costo-uterine smooth muscle and mesovarial leiomyoma formation *in vivo* by β-receptor agonists. *Arch Toxicol* 65(7):575-579.

Connor K, Howell J, Safe S, Chen I, Liu H, Berhane K, Sciarretta C, Zacharewski T. 1998. Failure of chloro-s-triazine-derived compounds to induce estrogenic respones in vivo and in vitro. In: Ballantine, LG, McFarland JE, Hackett DS, eds, *Triazine Herbicides: Risk Assessment (ACS Symposium Series No. 683)*, American Chemical Society, Washington, DC, USA, 424-431.

Cook JC, Frame SR, Obourn JD. 1997. Leydig Cell Tumors. In Boekleheide K, Chapin RE, Hoyer PB, Harris C, eds, *Comprehensive Toxicology, first edition, Volume 10, Reproductive and Endocrine Toxicology*, Pergamon, NY, USA, 10:193-201.

Cook JC, Klinefelter GR, Hardisty JF, Sharpe RM, Foster PMD. 1999. Rodent Leydig cell tumorigenesis: A review of the physiology, pathology, mechanisms, and relevance to humans. *Crit Rev Toxicol* 29(2):169-261.

Cook JC, Murray SM, Frame SR, Hurtt ME. 1992. Induction of Leydig cell adenomas by ammonium perfluorooctanoate: a possible endocrine-related mechanism. *Toxicol Appl Pharmacol* 113(2):209-217.

Cox D, O'Kennedy R, Thornes RD. 1989. The rarity of liver toxicity in patients treated with coumarin (1,2 benzopyrone). *Hum Toxicol* 8(6):501-506.

Cuervo AMK, Hildebrand H, Bomhard EM, Dice JF. 1999. Direct lysosomal uptake of α_2-microglobulin contributes to chemically induced nephropathy. *Kidney Intern* 55:529-545.

Curran PG, DeGroot LJ. 1991. The effect of hepatic enzyme-inducing drugs on thyroid hormones and the thyroid gland. *Endocrine Rev* 12:135-150.

Dexeus FH, Logothetis CJ, Sella A, Fitz K, Amato R, Reuben JM, Dozier N. 1990. Phase II study of coumarin and cimetidine in patients with metastatic renal cell carcinoma. *J Clin Oncol* 8:325-329.

ECETOC. 1992. Hepatic peroxisome proliferation. Monograph No. 17. *European Centre for Ecotoxicology and Toxicology of Chemicals*, Brussels, Belgium.

ECETOC. 2002. Recognition of, and differentiation between, adverse and non-adverse effects in toxicology studies. Technical Report No. 85. *European Centre for Ecotoxicology and Toxicology of Chemicals*, Brussels, Belgium.

ECETOC. 2003. Derivation of assessment factors for human health risk assessment. Technical Report No. 86. *European Centre for Ecotoxicology and Toxicology of Chemicals*, Brussels, Belgium.

Egan D, O'Kennedy R, Moran E, Cox D, Prosser, E, Thornes RD. 1990. The pharmacology, metabolism, analysis, and application of coumarin and coumarin-related compounds. *Drug Metab Rev* 22(5):503-529.

Eldridge JC, Fleenor-Heyser DG, Extrom PC, Wetzel LT, Breckenridge CB, Gillis JH, Luempert LG III, Stevens JT. 1994. Short-term effects of chlorotriazines on estrus in female Sprague-Dawley and Fischer 344 rats. *J. Toxicol Environ Health* 43:155-167.

Eldridge JC, McConnell RF, Wetzel LT, Tisdel MO. 1998. Appearance of mammary tumors in atrazine-treated female rats: probable mode of action involving strain-related control of ovulation and estrous cycling. In Ballantine LG, McFarland JE, Hackett DS, eds, *Triazine Herbicides: Risk Assessment (ACS Symposium Series No. 683)*, American Chemical Society, Washington, DC, USA, 414-423.

Ellis MK, Whitfield AC, Gowans LA, Auton TR, Provan WM, Lock EA, Smith LL. 1995. Inhibition of 4-hydroxyphenylpyruvate dioxygenase by 2-(2-nitro-4-trifluoromethylbenzoyl)-cyclohexane-1,3-dione and 2-(2-chloro-4-methanesulfonylbenzoyl)-cyclohexane-1,3-dione. *Toxicol Appl Pharmacol* 133(1):12-19.

EU Scientific Committee on Plants. 2001. Opinion of the scientific committee on plants regarding the inclusion of 2,4-dichlorophenoxyacetic acid (2,4-D) in annex I to council directive 91/414/EEC concerning the placing of plant protection products on the market.

Evans JG, Gaunt IF, Lake BG. 1979. Two-year toxicity study on coumarin in the baboon. *Food Cosmet Toxicol* 17(3):187-193.

Feron VJ, Bruyntjes JP, Woutersen RA, Immel HR, Appelman LM. 1988. Nasal tumours in rats after short-term exposure to a cytotoxic concentration of formaldehyde. *Cancer Lett* 39(1):101-111.

Foxworthy PS, White SL, Hoover DM, Eacho PI. 1990. Effect of ciprofibrate, bezafibrate, and LY171883 on peroxisomal β-oxidation in cultured rat, dog and rhesus monkey hepatocytes. *Toxicol Appl Pharmacol* 104(3):386-394.

Fukushima S, Arai M, Nakanowatari J, Hibino T, Okuda M, Ito N. 1983. Differences in susceptibility to sodium saccharin among various strains of rats and other animal species. *Gann* 74(1):8-20.

Gehring PJ, Betso JE. 1978. Phenoxy acids: Effects and fate in mammals. In Ramel C, ed, *Chlorinated phenoxy acids and their dioxins - Ecol Bull Stockholm* 27:122-133.

Goldsmith LA. 1983. Tyrosinemia and related disorders. In Stanbury JB, Wyngaarden JB, Fredrickson DS, Goldstein JL, Brown MS, eds, *The metabolic basis of inherited disease*, 5th ed - McGraw-Hill, New York, USA, pp 287-299.

Graves RJ, Coutts C, Green T. 1995. Methylene chloride-induced DNA damage: an interspecies comparison. *Carcinogen* 16(8):1919-1926.

Green T. 1997. Methylene chloride induced liver and lung tumours: an overview of the role of mechanistic studies in human safety assessment. *Hum Exp Toxicol* 16(1): 3-13.

Grice HC. 1988. Safety evaluation of butylated hydroxyanisole from the perspective of effects on forestomach and oesophageal squamous epithelium. *Food Chem Toxicol* 26(8):717-723.

Haber LT, Maier A, Zhao Q, Dollarhide JS, Savage RE, Dourson ML. 2001. Applications of mechanistic data in risk assessment: the past, present, and future. *Toxicol Sci* 61(1):32-39.

Hall MG, Wilks MF, Provan WM, Eksborg S, Lumholtz B. 2001. Pharmacokinetics and pharmacodynamics of NTBC (2-(2-nitro-4-fluoromethylbenzoyl)-1,3-cyclohexanedione) and mesotrione, inhibitors of 4-hydroxyphenyl pyruvate dioxygenase (HPPD) following a single dose to healthy male volunteers. *Br J Clin Pharmacol* 52(2):169-177.

Hard GC, Rodgers IS, Baetcke KP, Richards WL, McGaughy RE, Valcovic LR. 1993. Hazard evaluation of chemicals that cause accumulation of α2u-globulin, hyaline droplet nephropathy, and tubule neoplasia in the kidneys of male rats. *Environ Health Perspect* 99:313-349.

Heck HD, Casanova M, Starr TB. 1990. Formaldehyde toxicity - new understanding. *Crit Rev Toxicol* 20(6):397-426.

Henderson JM, Faraj BA, Ali FM, Rudman D. 1981. Tyrosine transaminase activity in normal and cirrhotic liver. *Dig Dis Sci* 26(2):124-128.

Hill AB. 1965. The environment and disease: association or causation? *Proc Royal Soc Med* 58:295-300.

Hill RN, Erdreich LS, Paynter OE, Roberts PA, Rosenthal SL, Wilkinson CF. 1989. Thyroid follicular cell carcinogenesis. *Toxicol Sci* 12(4):629-697.

Hirose M, Masuda A, Hasegawa R, Wada S, Ito N. 1990. Regression of butylated hydroxyanisole (BHA)-induced hyperplasia but not displasia in the forestomach of hamsters. *Carcinogenesis* 11(2):239-244.

IARC. 1995a. Wood dust and formaldehyde. IARC Monographs on the evaluation of carcinogenic risks to humans. Volume 62. *International Agency for Research on Cancer*, Lyons, France. http://monographs.arc.fr/htdocs/indexes/vol62index.html

IARC. 1995b. Peroxisome proliferation and its role in carcinogenesis. IARC Technical Report No. 24. *International Agency for Research on Cancer*, Lyons, France.

Ito N, Hirose M, Fukushima S, Tsuda H, Shirai T, Tatematsu M. 1986. Studies on antioxidants: their carcinogenic and modifying effects on chemical carcinogenesis. *Food Chem Toxicol* 24:1071-1082.

Iverson F. 1995. Phenolic antioxidants: health protection branch studies on butylated hydroxyanisole. *Cancer Lett* 93(1):49-54.

Jack D, Poynter D, Spurling NW. 1983. Beta-adrenoreceptor stimulants and mesovarian leiomyomas in the rat. *Toxicology* 27:315-320.

Kagawa M, Hakoi K, Yamamoto A, Futakuchi M, Hirose M. 1993. Comparison of reversibility of rat forestomach lesions induced by genotoxic and non-genotoxic carcinogens. *Jpn J Cancer Res* 84(11):1120-1129.

Kelly WA, Marler RJ, Weikel JH. 1993. Drug-induced mesovarial leiomyomas in the rat - A review and additional data. *J Am Coll Toxicol* 12(1):13-22.

Kerns WD, Pavkov KL, Donofrio DJ, Gralla EJ, Swenberg JA. 1983. Carcinogenicity of formaldehyde in rats and mice after long-term inhalation exposure. *Cancer Res* 43(9):4382-4392.

Kroes R, Wester PW. 1986. Forestomach carcinogens: possible mechanisms of action. *Food Chem Toxicol* 24(10-11):1083-1089.

Lake BG. 1995a. Peroxisome proliferation: Current mechanisms relating to non-genotoxic carcinogenesis. *Toxicol Lett* 82-83:673-681.

Lake BG. 1995b. Mechanisms of hepatocarcinogenicity of peroxisome-proliferating drugs and chemicals. *Annu Rev Pharmacol Toxicol* 35:483-507.

Lake BG. 1999. Coumarin metabolism, toxicity and carcinogenicity: Relevance for human risk assessment. *Food Chem Toxicol* 37(4):423-453.

Lake BG, Evans JG, Walters DG, Price RJ. 1990. Studies on the metabolism and hepatotoxicity of coumarin in the rat and Syrian hamster. *Hum Exp Toxicol* 9:356.

Lake BG, Gaudin H, Price RJ, Walters DG. 1992. Metabolism of [3-^{14}C] coumarin to polar and covalently bound products by hepatic microsomes from the rat, Syrian hamster, gerbil and humans. *Food Chem Toxicol* 30(2):105-115.

Lake BG, Grasso P. 1996. Comparison of the hepatotoxicity of coumarin in the rat, mouse, and Syrian hamster: a dose and time response study. *Fund Appl Toxicol* 34(1):105-117.

Lake BG, Gray TJ, Evans JG, Lewis DF, Beamand JA, Hue KL. 1989. Studies on the metabolism and hepatotoxicity of coumarin-induced toxicity in rat hepatocytes: comparison with dihydrocoumarin and other coumarin metabolites. *Toxicol Appl Pharmacol* 97(2):311-323.

Lappin GJ, Hardwick TD, Stow R, Pigott GH, van Ravenzwaay B. 2002. Absorption, metabolism and excretion of 4-chloro-2-methylphenoxyacetic acid (MCPA) in rat and dog. *Xenobiotica* 32(2):153-163.

Lehman-McKeeman LD, Rivera-Torres MI, Caudill D. 1990. Lysosomal degradation of α2u-globulin and α2u-globulin-xenobiotic conjugates. *Toxicol Appl Pharmacol* 103(3):539-548.

Libretto SE. 1994. A review of the toxicology of salbutamol (albuterol). *Arch Toxicol* 68(3):213-216.

Lock EA, Gaskin P, Ellis MK, Provan WM, Robinson M, Smith LL, Prisbylla MP, Mutter LC. 1996. Tissue distribution of 2-(2-nitro-4-trifluoromethylbenzoyl)cyclohexane-1,3-dione (NTBC): effect on enzymes involved in tyrosine catabolism and relevance to ocular toxicity in the rat. *Toxicol Appl Pharmacol* 141(2):439-447.

Lock EA, Gaskin P, Ellis MK, Provan WM, Robinson M, Smith LL. 2000. Tissue distribution of 2-(2-nitro-4-trifluoromethylbenzoyl)cyclohexane-1,3-dione (NTBC) and its effect on enzymes involved in tyrosine catabolism in the mouse. *Toxicology* 144(1):179-187.

Lutz U, Lugli S, Bitsch A, Schlatter J, Lutz WK. 1997. Dose response for the stimulation of cell division by caffeic acid in forestomach and kidney of the male F344 rat. *Toxicol Sci* 39(2):131-137.

MacInnes JI, Nozik ES, Kurtz DT. 1986. Tissue-specific expression of the rat alpha2u globulin gene family. *Mol Cell Biol* 6(10):3563-3567.

McClain RM, Levin AA, Posch R, Downing JC. 1989. The effect of phenobarbital on the metabolism and excretion of thyroxine in rats. *Toxicol Appl Pharmacol* 99(2):216-228.

McClain RM. 1992. Thyroid gland neoplasia: non-genotoxic mechanisms. *Toxicol Lett* 64-65:397-408.

McConnell EE. 1992. Thyroid follicular cell carcinogenesis: results from 343 2-year carcinogenicity studies conducted by the NCI/NTP. *Reg Toxicol Pharmacol* 16(2):177-188.

Mennear JH, McConnell EE, Huff JE, Renne RA, Giddens E. 1988. Inhalation toxicology and carcinogenesis studies of methylene chloride (dichloromethane) in F344/N rats and B6C3F_1 mice. *Ann NY Acad Sci* 534:343-351.

Moch RW. 1988. Forestomach lesions induced by butylated hydroxyanisole and ethylene dibromide: a scientific and regulatory perspective. *Toxicol Pathol* 16(2):172-183.

Monticello TM, Swenberg JA, Gross EA, Leininger JR, Kimbell JS, Seilkop S, Starr TB, Gibson JE, Morgan KT. 1996. Correlation of regional and nonlinear formaldehyde-induced nasal cancer with proliferating populations of cells. *Cancer Res* 56(5):1012-1022.

National Toxicology Program (NTP). 1993. Toxicology and carcinogenesis studies of coumarin (CAS No. 91-64-5) in F344/N rats and B6C3F1 mice (gavage studies). Technical report series No. 422. NIH Publication No. 92-3153. US Dept Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC, USA. http://ntp.niehs.nih.gov/ntp/htdocs/LT_rtps/tr422.pdf

Nelson LW, Kelly WA. 1971. Mesovarial leiomyomas in rats in a chronic toxicity study of soterenol hydrochloride. *Vet Path* 8:452-457.

Neuhaus OW. 1986. Renal reabsorption of low molecular weight proteins in adult male rats: α2u-globulin. *Proc Soc Exp Biol Med* 182:531-539.

O'Connor JC, Plowchalk DR, Van Pelt CS, Davis LG, Cook JC. 2000. Role of prolactin in chloro-S-triazine rat mammary tumorigenesis. *Drug Chem Toxicol* 23(4):575-601.

Olson MJ, Johnson JT, Reidy CA. 1990. A comparison of male rat and human urinary proteins: implications for human resistance to hyaline droplet nephropathy. *Toxicol Appl Pharmacol* 102(3):524-536.

Owen NV, Worth HM, Kiplinger GF. 1973. The effects of long-term ingestion of methimazole on the thyroids of rats. *Food Cosmet Toxicol* 11:649-653.

Paynter OE, Burin GJ, Jaeger RB, Gregorio CA. 1988. Goitrogens and thyroid follicular cell neoplasia: evidence for a threshold process. *Regul Toxicol Pharmacol* 8(1):102-119.

Powell CJ, Berry CL. 1999. Non-genotoxic or epigenetic carcinogenesis. In Ballantyne B, Marrs TC, Syversen T, eds, *General and Applied Toxicology*. Macmillan Reference Ltd, London, UK, 1119-1135.

Poynter D, Harris DM, Jack D. 1978. Salbutamol: lack of evidence of tumour induction in man. *Brit Med J* 1(6104):46-47.

Prescott-Mathews JS, Poet TS, Borghoff SJ. 1999. Evaluation of the *in vivo* interaction of methyl tert-butyl ether with α2u-globulin in male F-344 rats. *Toxicol Appl Pharmacol* 157(1):60-67.

Provan WM, Botham JW, Doe JE, Lewis RW, Robinson M. 1999. The mechanism of toxicity of a triketone herbicide. *Hum Exp Toxicol* 18:746.

Ratanasavanh D, Lamiable D, Biour M, Guedes Y, Gersberg M, Leutenegger E, Riche C. 1996. Metabolism and toxicity of coumarin on cultured human, rat, mouse and rabbit hepatocytes. *Fundam Clin Pharmacol* 10(6):504-510.

Renwick AG, Lazarus NR. 1998. Human variability and noncancer risk assessment - An analysis of the default uncertainty factor. *Reg Toxicol Pharmacol* 27(1):3-20.

Robinson M. 1995. The corneal effects of 2-(2-nitro-4-trifluoromethylbenzoyl)-cyclohexane-1,3-dione (NTBC) in the rat. In Weisse I, Hockwin O, Green K, Tripathi RC, eds. *Ocular Toxicology,* Plenum, New York / London, 327-334.

Roy AK, Neuhaus OW. 1966. Proof of the hepatic synthesis of a sex-dependent protein in the rat. *Biochim Biophys Acta* 127(1): 82-87.

Schlosser PM, Bogdanffy MS. 1999. Determining modes of action for biologically based risk assessment. *Reg Toxicol Pharmacol* 30(1):75-79.

Short BG, Burnett VL, Cox MG, Bus JS, Swenberg JA. 1987. Site-specific renal cytotoxicity and cell proliferation in male rats exposed to petroleum hydrocarbons. *Lab Invest* 57(5):564-577.

Simpkins JW, Eldridge JC, Wetzel LT. 1998. Role of strain-specific reproductive patterns in the appearance of mammary tumors in atrazine-treated rats. In Ballantine LG, McFarland J.E, Hackett DS, eds. *Triazine Herbicides: Risk Assessment (ACS Symposium Series No. 683).* American Chemical Society, Washington, DC, USA 399-413.

Sonich-Mullin C, Fielder R, Wiltse J, Baetcke K, Dempsey J, Fenner-Crisp P, Grant D, Hartley M, Knaap A, Kroese D, Mangelsdorf I, Meek E, Rice JM, Younes M. 2001. IPCS conceptual framework for evaluating a mode of action for chemical carcinogenesis. *Reg Toxicol Pharmacol* 34(2):146-152.

Squire RA. 1985. Histopathological evaluation of rat urinary bladders from the IRDC two-generation bioassay of sodium saccharin. *Food Chem Toxicol* 23(4-5):491-497.

Swenberg JA, Lehman-McKeeman LK. 1999. α_2-Urinary globulin-associated nephropathy as a mechanism of renal tubule cell carcinogenesis in male rats. In Capen CC, Dybing E, Rice JM, Wilbourn JD, eds. *Differences in Thyroid, Kidney and Urinary Bladder Carcinogenesis.* International Agency for Research on Cancer, Lyons, France. *IARC Sci Publ* 147:95-118.

Thakur AK, Wetzel LT, Voelker RW and Wakefield AE. 1998. Results of a two-year oncogenicity study in Fischer 344 rats with atrazine. In Ballantine LG, McFarland JE, Hackett DS, eds. *Triazine Herbicides: Risk Assessment (ACS Symposium Series No. 683)*. American Chemical Society, Washington, DC, USA, 384-398.

Timchalk C. 2004. Comparative inter-species pharmacokinetics of phenoxyacetic acid herbicides and related organic acids. Evidence that the dog is not a relevant species for evaluation of human health risk. *Toxicol* 200(1):1-19.

Timchalk C, Nolan RJ. 1997. Pharmacokinetics of triclopyr (3,5,6-trichloro-2-pyridinyloxyacetic acid) in the beagle dog and rhesus monkey: perspective on the reduced capacity of dogs to excrete this organic acid relative to the rat, monkey and human. *Toxicol Appl Pharmacol* 144(2):268-278.

Ueno I, Hirono I. 1981. Non-carcinogenic response to coumarin in Syrian golden hamsters. *Food Cosmet Toxicol* 19(3):353-355.

US EPA. 1991a. Risk Assessment Forum: Alpha2u-globulin: Association with chemically induced renal toxicity and neoplasia in the male rat. EPA/625/3-91/019F. *US Environmental Protection Agency*, Washington, DC, USA.

US EPA. 1991b. Formaldehyde risk assessment update - Final draft. Office of Toxic Substances, *US Environmental Protection Agency*, Washington, DC.

US EPA. 1996. Proposed guidelines for carcinogenic risk assessment. EPA/600/P-92/003C. Office of Research and Development, *US Environmental Protection Agency*, Washington, DC, USA.

US EPA. 1999. Guidelines for carcinogenic risk assessment. NCEA-F-0644. Review draft. *US Environmental Protection Agency*, Washington, DC, USA.

Van Raaij JA, Kaptein E, Visser TJ, van den Berg KJ. 1993. Increased glucuronidation of thyroid hormone in hexachlorobenzene-treated rats. *Biochem Pharmacol* 45(3):627-631.

van Ravenzwaay B, Hardwick TD, Needham D, Pethen S, Lappin GL. 2003. Comparative metabolism of 2,4-dichlorophenoxyacetic acid (2,4-D) in rat and dog. *Xenobiotica* 33(8):805-821.

Verhagen H, Schutte B, Reynders MMJ, Blijham GH, ten Hoor F, Kleinjans JCS. 1988. Effect of short-term dietary administration of butylated hydroxyanisole on cell kinetic parameters in rat gastro-intestinal tract, assessed by immunocytochemistry and flow cytometry. *Carcinogenesis* 9(6):1107-1109.

Villalobos AR, Dunnick CA, Pritchard JB. 1996. Mechanism mediating basolateral transport of 2,4-dichlorophenoxyacetic acid in rat kidney. *J Pharmacol Exp Ther* 278(2):582-589.

Wada N, Marsman DS, Popp JA. 1992. Dose-related effects of the hepatocarcinogen, Wy-14,643, on peroxisomes and cell replication. *Toxicol Sci* 18(1):149-154.

Wester PW, Kroes R. 1988. Forestomach carcinogens: pathology and relevance to man. *Toxicol Pathol* 16(2):165-171.

Wetzel LT, Luempert LG III, Breckenridge CB, Tisdel MO, Stevens JT, Thakur AK, Extrom PJ, Eldridge JC. 1994. Chronic effects of atrazine on estrous and mammary tumor formation in female Sprague-Dawley and Fischer 344 rats. *J Toxicol Environ Health* 43(2):169-182.

WHO. 1987. Principles for the Safety Assessment of Food Additives and Contaminants in Food. Environmental Health Criteria No 70. *World Health Organization*, Geneva, Switzerland.

Woutersen RA, van Garderen-Hoetmer A, Bruijntjes JP, Zwart A, Feron VJ. 1989. Nasal tumors in rats after severe injury to the nasal mucosa and prolonged exposure to 10 ppm formaldehyde. *J Appl Toxicol* 9(1):39-46.

MEMBERS OF THE TASK FORCE

F. Waechter (Chairman)	Syngenta CH - Basel
E. Bomhard	Bayer HealthCare D - Wuppertal
S. Borghoff	CIIT (now: Centers for Health Research) US - North Carolina
P.-G. Pontal	Bayer CropScience F - Sophia Antipolis
M. Provan	Syngenta UK - Macclesfield
M. Butler (Secretary) C. Hennes (Secretary)	ECETOC B - Brussels

Acknowledgement: Prof. I. Kimber (Syngenta) kindly reviewed the final version of the manuscript.

MEMBERS OF THE SCIENTIFIC COMMITTEE AT PEER REVIEW (Oct 2001)

B. Hildebrand (Chairman) Vice president, Experimental Toxicology and Ecology	BASF D - Ludwigshafen
E. Bomhard Head, Industrial Toxicology	Bayer D - Wuppertal
C. Braun Occupational Toxicologist	Akzo Nobel NL - Arnhem
P. Calow Professor of Zoology	University of Sheffield UK - Sheffield
N. Carmichael[a] (Co-Chairman) Head, Toxicology	Aventis Crop Science (now Bayer CropScience) F - Sophia Antipolis
C. d'Hondt Head, Environmental Safety Department	Syngenta CH - Basel
T. Feijtel Manager, Professional and Regulatory Services	Procter & Gamble, B - Brussels
H. Greim Director, Institute of Toxicology and Environmental Hygiene	Technical University München D - München
J. Jackson Senior associate, Medical Adviser	Monsanto B - Brussels
R. Millischer Head, Industrial Toxicology Department	Atofina (now Arkema group) F - Paris
G. Randall (Co-Chairman)	AstraZeneca UK - Brixham
A. Sarrif[a] Director, Toxicology Affairs, Europe	DuPont D - Bad Homburg
L. Smith Director, Central Toxicology Laboratory	Syngenta UK - Macclesfield
J. Solbé Head, SEAC Environment	Unilever UK - Bebington

G. Swaen Head, Occupational Epidemiology Unit	Maastricht University NL - Maastricht
B. van Ravenzwaay Director, Experimental Toxicology and Ecology	BASF D - Ludwigshafen
Dr. H.-J. Wiegand Head, Product Safety Department	Degussa D - Düsseldorf

MEMBERS OF THE SCIENTIFIC COMMITTEE AT PUBLICATION

G. Randall (Chairman) Consultant	UK - Stoke Gabriel
R. Bars Toxicology Research	Bayer CropScience F - Sophia Antipolis
P. Calow Director	Environmental Assessment Inst. DK - Copenhagen
W. de Wolf Director of Health and Environmental Sciences	DuPont B - Mechelen
J. Doe Head of Health Assessment	Syngenta CTL UK - Macclesfield
A. Flückiger Head of Corporate Health Protection	F. Hoffmann - La Roche CH - Basel
H. Greim Director, Institute of Toxicology and Environmental Hygiene	Technical University München D - München
T. Hutchinson Principal Scientist, Global Safety Assessment	AstraZeneca S - Södertälje
C. Money Industrial Hygiene Adviser, Europe	ExxonMobil B - Brussels
D. Owen Regulatory and Science Issues Manager	Shell UK - London
G. Swaen Epidemiologist, Epidemiology, Health Services	Dow NL - Terneuzen

B. van Ravenzwaay[a]
Director, Experimental Toxicology and Ecology

BASF
D - Ludwigshafen

H.-J. Wiegand
Product Stewardship, Corporate Environment, Safety, Health, Quality

Degussa
D - Düsseldorf

[a] Stewards: Dr. N. Carmichael (Bayer CropScience); resigned from the Scientific Committee on 31.12.2003.
Dr. A. Sarrif (DuPont); resigned from the Scientific Committee on 31.12.2004.
Dr. B. van Ravenzwaay acted as Steward for publication.

ECETOC PUBLISHED REPORTS

Monographs

No. Title

No. 1 Good Laboratory Practice (Published October 1979)
No. 2 A Contribution to Strategy for Identification and Control of Occupational Carcinogens (Published September 1980)
No. 3 Risk Assessment of Occupational Chemical Carcinogens (Published May 1985)
No. 4 Hepatocarcinogenesis in Laboratory Rodents: Relevance for Man (Published October 1982)
No. 5 Identification and Assessment of the Effects of Chemicals on Reproduction and Development (Reproductive Toxicology) (Published December 1983)
No. 6 Acute Toxicity Tests, LD_{50} (LC_{50}) Determinations and Alternatives (Published May 1985)
No. 7 Recommendations for the Harmonisation of International Guidelines for Toxicity Studies (Published December 1985)
No. 8 Structure-Activity Relationships in Toxicology and Ecotoxicology: An Assessment (Summary) (Published June 1986)
No. 9 Assessment of Mutagenicity of Industrial and Plant Protection Chemicals (Published June 1987)
No. 10 Identification of Immunotoxic Effects of Chemicals and Assessment of their Relevance to Man (Published August 1987)
No. 11 Eye Irritation Testing (Published June 1988)
No. 12 Alternative Approaches for the Assessment of Reproductive Toxicity (with emphasis on embryotoxicity/teratogenicity) (Published November 1989)
No. 13 DNA and Protein Adducts: Evaluation of their Use in Exposure Monitoring and Risk Assessment (Published October 1989)
No. 14 Skin Sensitisation Testing (Published March 1990)
No. 15 Skin Irritation (Published July 1990)
No. 16 Early Indicators of Non-Genotoxic Carcinogenesis (Published June 1991)
No. 17 Hepatic Peroxisome Proliferation (Published May 1992)
No. 18 Evaluation of the Neurotoxic Potential of Chemicals (Published September 1992)
No. 19 Respiratory Allergy (Published August 1993)
No. 20 Percutaneous Absorption (Published August 1993)
No. 21 Immunotoxicity: Hazard Identification and Risk Characterisation (Published September 1994)
No. 22 Evaluation of Chemicals for Oculotoxicity (Published November 1994)
No. 23 Receptor Mediated Mechanisms in Chemical Carcinogenesis (Published December 1995)
No. 24 Risk Assessment for Carcinogens (Published July 1996)
No. 25 Practical Concepts for Dose Selection in Chronic Toxicity and Carcinogenicity Studies in Rodents (Published February 1996)
No. 26 Aquatic Toxicity Testing of Sparingly Soluble Volatile and Unstable Substances (Published September 1996)
No. 27 Aneuploidy (Published August 1997)
No. 28 Dose-response and threshold-mediated mechanisms in mutagenesis - Mutation Research Special Issue (Published January 2000)
No. 29 Skin Sensitisation Testing for the Purpose of Hazard Identification and Risk Assessment (Published September 2000)
No. 30 Genetic Susceptibility to Environmental Toxicants (Published October 2001)
No. 31 Guidance on Evaluation of Reproductive Toxicity Data (Published February 2002)
No. 32 Use of Human Data in Hazard Classification for Irritation and Sensitisation (Published July 2002)
No. 33 Application of Physiological - Toxicokinetic Modelling to Health Hazard Assessment of Chemical Substances (Published February 2003)
No. 34 Toxicogenomics in Genetic Toxicology and Hazard Determination (Published July 2005)

Technical Reports

No. Title

No. 1 Assessment of Data on the Effects of Formaldehyde on Humans (updated by TR No. 6) (Published January 1979)
No. 2 The Mutagenic and Carcinogenic Potential of Formaldehyde (Published May 1981)
No. 3 Assessment of Test Methods for Photodegradation of Chemicals in the Environment (Published August 1981)
No. 4 The Toxicology of Ethylene Glycol Monoalkyl Ethers and its Relevance to Man (updated by TR No. 17) (Published June 1982)
No. 5 Toxicity of Ethylene Oxide and its Relevance to Man (Published September 1982)
No. 6 Formaldehyde Toxicology: An Up-Dating of ECETOC Technical Reports 1 and 2 (Published September 1982)
No. 7 Experimental Assessment of the Phototransformation of Chemicals in the Atmosphere (Published September 1983)
No. 8 Biodegradation Testing: An Assessment of the Present Status (Published November 1983)
No. 9 Assessment of Reverse-Phase Chromatographic Methods for Determining Partition Coefficients (Published December 1983)
No. 10 Considerations Regarding the Extrapolation of Biological Data in Deriving Occupational Exposure Limits (Published February 1984)
No. 11 Ethylene Oxide Toxicology and its Relevance to Man: An Up-Dating of ECETOC Technical Report No. 5 (Published March 1984))
No. 12 The Phototransformation of Chemicals in Water: Results of a Ring-Test (Published June 1984)
No. 13 The EEC 6th Amendment: A Guide to Risk Evaluation for Effects on the Environment (Published March 1984)
No. 14 The EEC 6th Amendment: A Guide to Risk Evaluation for Effects on Human Health (Published March 1984)
No. 15 The Use of Physical-Chemical Properties in the 6th Amendment and their Required Precision, Accuracy and Limiting Values (Published June 1984)
No. 16 A Review of Recent Literature on the Toxicology of Benzene (Published December 1984)
No. 17 The Toxicology of Glycol Ethers and its Relevance to Man: An Up-Dating of ECETOC Technical Report No. 4 (updated by TR No. 64) (Published April 1985)
No. 18 Harmonisation of Ready Biodegradability Tests (Published April 1985)
No. 19 An Assessment of Occurrence and Effects of Dialkyl-o-Phthalates in the Environment (Published May 1985)
No. 20 Biodegradation Tests for Poorly-Soluble Compounds (Published February 1986)
No. 21 Guide to the Classification of Carcinogens, Mutagens, and Teratogens under the 6th Amendment (Published February 1986)
No. 22 Classification of Dangerous Substances and Pesticides in the EEC Directives. A Proposed Revision of Criteria for Inhalational Toxicity (Published January 1987)
No. 23 Evaluation of the Toxicity of Substances to be Assessed for Biodegradability (Published November 1986)
No. 24 The EEC 6th Amendment: Prolonged Fish Toxicity Tests (Published October 1986)
No. 25 Evaluation of Fish Tainting (Published January 1987)
No. 26 The Assessment of Carcinogenic Hazard for Human Beings exposed to Methylene Chloride (Published January 1987)
No. 27 Nitrate and Drinking Water (Published January 1988)
No. 28 Evaluation of Anaerobic Biodegradation (Published June 1988)
No. 29 Concentrations of Industrial Organic Chemicals Measured in the Environment: The Influence of Physico-Chemical Properties, Tonnage and Use Patterns (Published June 1988)
No. 30 Existing Chemicals: Literature Reviews and Evaluations (Fifth Edition) (No longer available) (Published May 1994)
No. 31 The Mutagenicity and Carcinogenicity of Vinyl Chloride: A Historical Review and Assessment (Published July 1988)
No. 32 Methylene Chloride (Dichloromethane): Human Risk Assessment Using Experimental Animal Data (Published May 1988)

No. 33 Nickel and Nickel Compounds: Review of Toxicology and Epidemiology with Special Reference to Carcinogenesis (Published February 1989)
No. 34 Methylene Chloride (Dichloromethane): An Overview of Experimental Work Investigating Species Differences in Carcinogenicity and their Relevance to Man (Published March 1989)
No. 35 Fate, Behaviour and Toxicity of Organic Chemicals Associated with Sediments (Published January 1990)
No. 36 Biomonitoring of Industrial Effluents (Published April 1990)
No. 37 Tetrachlorethylene: Assessment of Human Carcinogenic Hazard (Published May 1990)
No. 38 A Guide to the Classification of Preparations Containing Carcinogens, Mutagens and Teratogens (Published July 1990)
No. 39 Hazard Assessment of Floating Chemicals After an Accidental Spill at Sea (Published July 1990)
No. 40 Hazard Assessment of Chemical Contaminants in Soil (Published April 1992)
No. 41 Human Exposure to N-Nitrosamines, their Effects and a Risk Assessment for N-Nitrosodiethanolamine in Personal Care Products (Published August 1990)
No. 42 Critical Evaluation of Methods for the Determination of N-Nitrosamines in Personal Care and Household Products (Published February 1991)
No. 43 Emergency Exposure Indices for Industrial Chemicals (Published March 1991)
No. 44 Biodegradation Kinetics (Published September 1991)
No. 45 Nickel, Cobalt and Chromium in Consumer Products: Allergic Contact Dermatitis (Published March 1992)
No. 46 EC 7th Amendment: Role of Mammalian Toxicokinetic and Metabolic Studies in the Toxicological Assessment of Industrial Chemicals
(Published May 1992)
No. 47 EC 7th Amendment "Toxic to Reproduction": Guidance on Classification (Published August 1992)
No. 48 Eye Irritation: Reference Chemicals Data Bank (Second Edition) (Published June 1998)
No. 49 Exposure of Man to Dioxins: A Perspective on Industrial Waste Incineration (Published December 1992)
No. 50 Estimating Environmental Concentrations of Chemicals using Fate and Exposure Models (Published November 1992)
No. 51 Environmental Hazard Assessment of Substances (Published January 1993)
No. 52 Styrene Toxicology Investigation on the Potential for Carcinogenicity (Published August 1992)
No. 53 DHTDMAC: Aquatic and Terrestrial Hazard Assessment (CAS No. 61789-80-8) (Published February 1993)
No. 54 Assessment of the Biodegradation of Chemicals in the Marine Environment (Published August 1993)
No. 55 Pulmonary Toxicity of Polyalkylene Glycols (Published December 1997)
No. 56 Aquatic Toxicity Data Evaluation (Published December 1993)
No. 57 Polypropylene Production and Colorectal Cancer (Published February 1994)
No. 58 Assessment of Non-Occupational Exposure to Chemicals (Published May 1994)
No. 59 Testing for Worker Protection (Published April 1994)
No. 60 Trichloroethylene: Assessment of Human Carcinogenic Hazard (Published May 1994)
No. 61 Environmental Exposure Assessment (Published September 1994)
No. 62 Ammonia Emissions to Air in Western Europe (Published July 1994)
No. 63 Reproductive and General Toxicology of some Inorganic Borates and Risk Assessment for Human Beings (Published February 1995)
No. 64 The Toxicology of Glycol Ethers and its Relevance to Man (Published August 1995)
No. 65 Formaldehyde and Human Cancer Risks (Published May 1995)
No. 66 Skin Irritation and Corrosion: Reference Chemicals Data Bank (Published March 1995)
No. 67 The Role of Bioaccumulation in Environmental Risk Assessment: The Aquatic Environment and Related Food Webs (Published October 1995)
No. 68 Assessment Factors in Human Health Risk Assessment (updated by TR No. 86) (Published August 1995)
No. 69 Toxicology of Man-Made Organic Fibres (Published April 1996)
No. 70 Chronic Neurotoxicity of Solvents (Published February 1996)

No. 71 Inventory of Critical Reviews on Chemicals (Only available to ECETOC members) (Published August 1996)
No. 72 Methyl *tert*-Butyl Ether (MTBE) Health Risk Characterisation (Published June 1997)
No. 73 The Value of Aquatic Model Ecosystem Studies in Ecotoxicology (Published December 1997)
No. 74 QSARs in the Assessment of the Environmental Fate and Effects of Chemicals (Published June 1998)
No. 75 Organophosphorus Pesticides and Long-term Effects on the Nervous System (Published December 1998)
No. 76 Monitoring and Modelling of Industrial Organic Chemicals, with Particular Reference to Aquatic Risk Assessment (Published January 1999)
No. 77 Skin and Respiratory Sensitisers: Reference Chemicals Data Bank (Published August 1999)
No. 78 Skin Sensitisation Testing: Methodological Considerations (Published December 1999)
No. 79 Exposure Factors Sourcebook for European Populations (with Focus on UK Data) (Published June 2001)
No. 80 Aquatic Toxicity of Mixtures (Published July 2001)
No. 81 Human Acute Intoxication from Monochloroacetic Acid: Proposals for Therapy (Published November 2001)
No. 82 Risk Assessment in Marine Environments (Published December 2001)
No. 83 The Use of T25 Estimates and Alternative Methods in the Regulatory Risk Assessment of Non-threshold Carcinogens in the European Union (Published December 2002)
No. 84 Scientific Principles for Soil Hazard Assessment of Substances (Published July 2002)
No. 85 Recognition of, and Differentiation between, Adverse and Non-adverse Effects in Toxicology Studies (Published December 2002)
No. 86 Derivation of Assessment Factors for Human Health Risk Assessment (Published February 2003)
No. 87 Contact Sensitisation: Classification According to Potency (Published April 2003)
No. 88 Environmental Risk Assessment of Difficult Substances (Published June 2003)
No. 89 (Q)SARS: Evaluation of the Commercially Available Software for Human Health and Environmental Endpoints with Respect to Chemical Management Applications (Published September 2003)
No. 90 Persistence of Chemicals in the Environment (Published October 2003)
No. 91 Aquatic Hazard Assessment II (Published November 2003)
No. 92 Soil and Sediment Risk Assessment (Published December 2004)
No. 93 Targeted Risk Assessment (Published December 2004)
No. 94 Whole Effluent Assessment (Published December 2004)
No. 95 The Toxicology of Glycol Ethers and its Relevance to Man (Fourth Edition) Volume I and Volume II Substance Profiles (Published February 2005)
No. 96 Trends in Children's Health and the Role of Chemicals: State of the Science Review (Published June 2005)
No. 97 Alternative Testing Approaches in Environmental Safety Assessment (Published December 2005)
No. 98 Risk Assessment of PBT Chemicals (Published December 2005)

Joint Assessment of Commodity Chemicals (JACC) Reports

No. Title

No. 1 Melamine (Published February 1983)
No. 2 1,4-Dioxane (Published February 1983)
No. 3 Methyl Ethyl Ketone (Published February 1983)
No. 4 Methylene Chloride (Published January 1984)
No. 5 Vinylidene Chloride (Published August 1985)
No. 6 Xylenes (Published June 1986)
No. 7 Ethylbenzene (Published August 1986)
No. 8 Methyl Isobutyl Ketone (Published May 1987)

No. 9 Chlorodifluoromethane (Published October 1989)
No. 10 Isophorone (Published September 1989)
No. 11 1,2-Dichloro-1,1-difluoroethane (HFA-132b) (Published May 1990)
No. 12 1-Chloro-1,2,2,2-tetrafluoroethane (HFA-124) (Published May 1990 and updated by JACC No. 25 and No. 46)
No. 13 1,1-Dichloro-2,2,2-trifluoroethane (HFA-123) (Published May 1990 and updated by JACC No. 33)
No. 14 1-Chloro-2,2,2-trifluoromethane (HFA-133a) (Published August 1990)
No. 15 1-Fluoro 1,1-dichloroethane (HFA-141b) (Published August 1990 and updated by JACC No. 29)
No. 16 Dichlorofluoromethane (HCFC-21) (Published August 1990)
No. 17 1-Chloro-1,1-difluoroethane (HFA-142b) (Published August 1990)
No. 18 Vinyl Acetate (Published February 1991)
No. 19 Dicyclopentadiene (CAS: 77-73-6) (Published July 1991)
No. 20 Tris-/Bis-/Mono-(2 ethylhexyl) phosphate (Published May 1992)
No. 21 Tris-(2-butoxyethyl)-phosphate (CAS:78-51-3) (Published March 1992)
No. 22 Hydrogen Peroxide (CAS: 7722-84-1) (Published January 1993)
No. 23 Polycarboxylate Polymers as Used in Detergents (Published November 1993)
No. 24 Pentafluoroethane (HFC-125) (CAS: 354-33-6) (Published May 1994)
No. 25 1-Chloro-1,2,2,2-tetrafluoroethane (HCFC 124) (CAS No. 2837-89-0) (Second Edition) (Published July 1994 and updated by JACC 46)
No. 26 Linear Polydimethylsiloxanes (CAS No. 63148-62-9) (Published September 1994)
No. 27 *n*-Butyl Acrylate (CAS No. 141-32-2) (Published August 1994)
No. 28 Ethyl Acrylate (CAS No. 140-88-5) (Published September 1994)
No. 29 1,1-Dichloro-1-fluoroethane (HCFC-141b) (CAS No. 1717-00-6) (Published December 1994)
No. 30 Methyl Methacrylate (CAS No. 80-62-6) (Published February 1995 and updated by JACC No. 50)
No. 31 1,1,1,2-Tetrafluoroethane (HFC-134a) (CAS No. 811-97-2) (Published February 1995)
No. 32 Difluoromethane (HFC-32) (CAS No. 75-10-5) (Published May 1995)
No. 33 1,1-Dichloro-2,2,2-trifluoroethane (HCFC-123) (CAS No. 306-83-2) (Published February 1996 and updated by JACC No. 47)
No. 34 Acrylic Acid (CAS No. 79-10-7) (Published September 1995)
No. 35 Methacrylic Acid (CAS No. 79-41-4) (Published May 1996)
No. 36 *n*-Butyl Methacrylate; Isobutyl Methacrylate (CAS No. 97-88-1) (CAS No. 97-86-9) (Published December 1996)
No. 37 Methyl Acrylate (CAS No. 96-33-3) (Published September 1998)
No. 38 Monochloroacetic Acid (CAS No. 79-11-8) and its Sodium Salt (CAS No. 3926-62-3) (Published June 1999)
No. 39 Tetrachloroethylene (CAS No. 127-18-4) (Published December 1999)
No. 40 Peracetic Acid (CAS No. 79-21-0) and its Equilibrium Solutions (Published January 2001)
No. 41 *n*-Butanol (CAS No. 71-36-3) (Published March 2004)
No. 42 Tetrafluoroethylene (CAS No. 116-14-3) (Published December 2003)
No. 43 *sec*-Butanol (CAS No. 78-92-2) (Published March 2004)
No. 44 1, 1, 1, 3, 3-Pentafluoropropane (HFC-245fa) (Published June 2004)
No. 45 1, 1-Difluoroethane (HFC-152a) (CAS No. 75-37-6) (Published September 2004)
No. 46 1-Chloro-1,2,2,2-tetrafluoroethane (HCFC 124) CAS No. 2837-89-0 (Second Edition) (Published November 2004)
No. 47 1,1-Dichloro-2,2,2-trifluoroethane (HCFC-123) CAS No. 306-83-2 (Third Edition) (Published May 2005)
No. 48 Hexafluoropropylene (HFP) CAS No. 116-15-4 (Published September 2005)
No. 49 Vinylidene Fluoride CAS No. 75-38-7 (Published November 2005)
No. 50 1,1,1,2-Tetrafluoroethane (HFC-134a) (CAS No. 811-97-2) (Second Edition) (Published January 2006)

Special Reports

No.	Title
No. 8	HAZCHEM; A Mathematical Model for Use in Risk Assessment of Substances (Published October 1994)
No. 9	Styrene Criteria Document (Published June 1995)
No. 10	Hydrogen Peroxide OEL Criteria Document (CAS No. 7722-84-1) (Published July 1996)
No. 11	Ecotoxicology of some Inorganic Borates (Published March 1997)
No. 12	1,3-Butadiene OEL Criteria Document (Second Edition) (CAS No. 106-99-0) (Published January 1997)
No. 13	Occupational Exposure Limits for Hydrocarbon Solvents (Published August 1997)
No. 14	*n*-Butyl Methacrylate and Isobutyl Methacrylate OEL Criteria Document (Published May 1998)
No. 15	Examination of a Proposed Skin Notation Strategy (Published September 1998)
No. 16	GREAT-ER User Manual (Published March 1999)
No. 17	Risk Assessment Report for Existing Substances Methyl *tertiary*-Butyl Ether (Published December 2003)

Documents

No.	Title
No. 32	Environmental Oestrogens: Male Reproduction and Reproductive Development (Published January 1996)
No. 33	Environmental Oestrogens: A Compendium of Test Methods (Published July 1996)
No. 34	The Challenge Posed by Endocrine-disrupting Chemicals (Published February 1996)
No. 35	Exposure Assessment in the Context of the EU Technical Guidance Documents on Risk Assessment of Substances (Published May 1997)
No. 36	Comments on OECD Draft Detailed Review Paper: Appraisal of Test Methods for Sex-Hormone Disrupting Chemicals (Published August 1997)
No. 37	EC Classification of Eye Irritancy (Published December 1997)
No. 38	Wildlife and Endocrine Disrupters: Requirements for Hazard Identification (Published January 1998)
No. 39	Screening and Testing Methods for Ecotoxicological Effects of Potential Endocrine Disrupters: Response to the EDSTAC Recommendations and a Proposed Alternative Approach (Published January 1999)
No. 40	Comments on Recommendation from Scientific Committee on Occupational Exposure Limits for 1,3-Butadiene (Published October 2000)
No. 41	Persistent Organic Pollutants (POPs) Response to UNEP/INC/CEG-I Annex 1 (Published January 2000)
No. 42	Genomics, Transcript Profiling, Proteomics and Metabonomics (GTPM). An Introduction (Published April 2001)
No. 43	Contact Sensitisation: Classification According to Potency. A Commentary (Published July 2003)
No. 44	Guidance for the Interpretation of Biomonitoring Data (Published November 2005)

Workshop Reports

No.	Title
No. 1	Workshop on Availability, Interpretation and Use of Environmental Monitoring Data 20-21 March 2003, Brussels (Published December 2003)
No. 2	Strategy Report on Challenges, Opportunities and Research needs arising from the Definition, Assessment and Management of Ecological Quality Status as required by the EU Water Framework Directive based on the workshop EQS and WFD versus PNEC and REACH - are they doing the job? 27-28 November 2003, Budapest (Published March 2004)
No. 3	Workshop on the Use of Human Data in Risk Assessment 23-24 February 2004, Cardiff (Published November 2004)
No. 4	Influence of Maternal Toxicity in Studies on Developmental Toxicity 2 March 2004, Berlin (Published October 2004)
No. 5	Workshop on Alternative Testing Approaches in Environmental Risk Assessment 7-9 July 2004, Paris (Published December 2004)
No. 6	Workshop on Chemical Pollution, Respiratory Allergy and Asthma 16-17 June 2005, Leuven (Published December 2005)

Published Articles

2005 A rule-based screening environmental risk-assessment tool derived from EUSES.
Chemosphere 58 (2005) 1169-1176

2005 The ECETOC approach to targeted environmental risk assessment.
Environmental Toxicology and Chemistry, Vol. 24 No 2. pp 251-252, 2005

2005 Mode of action and aquatic exposure thresholds of no concern.
Environmental Toxicology and Chemistry, Vol. 24 No 2. pp 479-485, 2005

2005 Workshop: Influence of maternal toxicity in studies on developmental toxicity.
Regulatory Toxicology and Pharmacology 43 (2005) 114-115

2003 Human inhalation exposure to ethylene glycol.
Toxicokinetics and Metabolism.
Arch. Toxicol (2003) 77: 425-432

2003 Linear Alkylbenzene sulfonate and boron monitoring in four catchments in the UK contribution to GREAT-ER project # 11.
The Science of the Total Environment, 314/315 (2003) 271-288

2002 Recognition of adverse and nonadverse effects in toxicity studies.
Toxicology Pathology, Vol. 30, No. 1, pp. 66-74, 2002

2001 Skin sensitisation testing in potency and risk assessment.
Toxicological Sciences 59, 198-208 (2001)

2001 Skin sensitisation: New perspectives and recommendations.
Food and Chemical Toxicology 39 (2001) 293-301

2001 Scientific analysis of the proposed uses of T25 dose descriptor in chemical carcinogen regulation. An ECETOC Workshop Overview.
Regulatory Toxicology (2001)75:507 512

2000 ECETOC – EEMS (European Environmental Mutagen Society) Symposium on dose-response and threshold-mediated mechanisms in mutagenesis.
Salzburg, Austria: 7 September 1998.
General Introduction
Mutation Research 464 (2000) 1-2

2000 Monitoring, modelling and environmental exposure assessment of industrial chemicals in the aquatic environment.
Chemosphere 41 (2000) 1799-1808

2000 Sources of chemical contaminants and routes into the freshwater environment.
Food and Chemical Toxicology 38 (2000) S21-S27

2000 Ecological risk assessment of endocrine disrupters.
Environmental Health Perspectives, Vol. 108, No. 11, November 2000

2000 Removal of linear alkylbenzene sulfonate from a small Yorkshire stream: Contribution to GREAT-ER project # 7.
The Science of the Total Environment, 251/252 (2000) 265-275

1999 Eye irritation: Updated reference chemicals data bank.
Toxicology in vitro 13 (1999) 505-210

1998 Establishment of a test guideline for the evaluation of fish testing. Chemosphere (1988) 4:751-765

1998 UK monitoring study on the removal of linear alkylbenzene sulfonate in trickling filter type sewage treatment plants. Contribution to GREAT-ER project # 2.
The Science of the Total Environment, 210/211 (1988) 255-269

1998 Analysis of the ECETOC aquatic toxicity (EAT) database V: The relevance of Daphnia Magna as a representative test species.
Chemosphere, Vol. 36, No. 1, pp. 155-166, 1998

1998 Aneuploidy: A report of an ECETOC task force.
Mutation Research, 410 (1998) 3-79
(See also Monograph 27)

1998 Common mechanisms of toxicity: A case study of organophosphorus pesticides.
Toxicological Sciences, 41, 8-20 (1998)

1995 Pathology considerations for, and subsequent risk assessment of, chemicals identified as immunosuppressive in routine toxicology.
Fd. Chem. Toxic. Vol. 33, No. 3., pp. 239-243, 1995

1995 Use of environmental fate models in the risk assessment of substances.
Toxicology Modelling, Vol. 1, No. 1, 1995

1995 Polypropolene production and colorectal cancer: A review of the epidemiological evidence.
Occup. Med. Vol. 45, No. 2, pp. 67-74, 1995

1995 The role of ECETOC in the European Chemical Industry.
Spotlight, CEFIC Information Bulletin, September 1995

1994 Respiratory Allergy: Hazard identification and risk assessment.
Fundamental and Applied Toxicology 23, 145-158 (1994)

1994 Evaluation of the neurotoxic potential of chemicals in animals.
Fd Chem. Toxic. Vol. 32, No. 7, pp. 665-669, 1994
(See also Monograph 18)

1993 Nickel cobalt and chromium in consumer products: A role in allergic contact dermatitis?
Contact Dermatitis (1993) 28:15-25
(See also ECETOC Technical Report 45)

1991 Skin sensitisation: A critical review of predictive test methods in animals and man.
Fd Chem. Toxic. Vol. 29, No. 4, pp. 275-286, 1991

1989 Eye irritation: An ECETOC overview.
J. Toxicology, Cut & Ocuvar (1989) 8:51-57