Influence of Maternal Toxicity in Studies on Developmental Toxicity 2 March 2004, Berlin

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LRI
The Long-range Research initiative

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Influence of Maternal Toxicity in Studies on Developmental Toxicity

CONTENTS

1. EXECUTIVE SUMMARY

The Workshop was convened to explore whether the criteria to assess the influence of toxic effects induced in the maternal organism on the development of the embryo/foetus could be improved for classification purposes. Several conclusions and recommendations were made:

1. Regulatory classification of chemicals that have caused signs of developmental toxicity relies on a hazard-based approach. It does not take full account of exposure conditions during normal handling and use.
2. The currently practiced European classification of chemicals that have shown evidence of developmental toxicity does not provide a clear distinction between developmental effects that occur directly on the embryo/foetus (primary effect) and those that are associated with chemically-induced perturbations of maternal homeostasis (secondary effect). The appropriate consideration of such effects has profound ramifications for the classification of chemicals.
3. Relevance of route and mode of administration of test chemicals, dose-response data as well as toxicokinetics and toxicodynamics were recognised as important criteria that should be considered in the study design. The existing data are compelling, and one can no longer ignore the scientific evidence that the biological and toxicological effects differ profoundly depending on how the chemical enters into the body.
4. The use of expanded end points that are more sensitive to detect chemically induced maternal toxicity than those specified in the OECD 414 test guideline received endorsement from a majority of the participants. However, reservations from representatives of regulatory agencies meant that no true consensus could be reached. An experimental way forward to better define maternal and developmental toxicity in OECD 414 studies received broad support. New data with refined end points to detect the onset of maternal toxicity might improve the interpretation of the results of such studies and might provide a scientific basis for industry and regulators when making decisions leading to classification.
5. The majority of participants agreed with the recommendation to make the regulatory process reflect anticipated human exposure. This would mean that risk characterisation, as well as hazard identification, would become an important component in the classification of chemicals.

2. WORKSHOP OVERVIEW

Background

Maternal toxicity often occurs as a consequence of high doses required in developmental toxicity studies conducted according to current testing guidelines. Such maternal toxicity is likely to produce indirect effects on the progeny, for example through compromised placental circulation, which can be as consequential as effects detected from primary developmental toxicants. However, the effects of maternal toxicity need to be recognised as such, and distinguished from primary developmental toxicity.

The reason for holding this Workshop was a response to a Cefic's Long-range Research Initiative (LRI) members' survey. The LRI commissioned ECETOC to organise and report on the Workshop.

All invited participants were provided with a Background Paper (attached) in advance of the Workshop meeting. The purpose of that document was to give an overview of the state of the science and provide the rationale for focusing the meeting on the OECD 414 developmental toxicity guideline-compliant study in rats.

Objective of the Workshop

The general objective of the Workshop was to build the grounds for finding consensus on the criteria for an appropriate interpretation of developmental effects occurring in the presence of maternal toxicity. The aim was to put strong emphasis on defining the actions needed to develop a better insight into the genuine incidence of such secondary effects. This new knowledge would be included in future study design, so that phenomena of maternal toxicity could be identified and distinguished from primary developmental toxicity, and would thereby lead to a more reliable hazard identification with impact on classification and labelling.

Workshop Structure

The Workshop was an interactive limited scale event with 42 invited participants, representing a balanced participation from regulatory agencies, academia and industry. The morning session of the Workshop started with four individual presentations (abstracts attached).

In the afternoon, Workshop participants separated into three breakout groups to address in more depth three topics (see specifics below) that built on the preceding individual presentations of the morning session.

Plenary Lectures

The opening presentation by Dr. Welsch reviewed the present OECD 414 test guidelines and their recorded end points. Equivocal outcomes of developmental toxicity studies carried out according to OECD 414 at times cause differences in interpretation by study directors and regulatory agencies. In those cases, the testing observations do not allow an unequivocal decision as to whether developmental toxicity encountered at doses that induced maternal toxicity was the result of direct effects of the test article on the embryo/foetus (a primary effect) or may have been caused indirectly by toxic effects on the maternal organism (a secondary effect). The colloquial term "chemicals of the grey zone" was introduced and used repeatedly during subsequent Workshop discussions. In particular that designation was applied in the context of the deliberations concerning expansion of the end points of maternal toxicity assessments with a small number of chemicals for which no unequivocal decision could be made as to a primary or secondary effect causing developmental toxicity.

In the second presentation, Dr. Sullivan provided a historical perspective as to how the classification of chemicals for toxicity to reproduction was introduced in 1992 and how since then it has worked in practice. In the intervening more than 10 years of regulatory agency application of the Dangerous Substances Directive 69/549/EEC, it has become apparent that a hazard-based approach used in the interpretation of animal testing data has been strictly applied. This has caused classification and labelling of too many chemicals into categories that have had serious economic consequences for the manufacturers and no benefits to public health. There are now several initiatives underway in various expert working groups of the EU how to improve the interpretation of animal study data and the resulting regulatory approach. Dr. Sullivan concluded on the recommendation that a weight of evidence approach is necessary so that more attention is given to risk characterisation. The hazard identification-based classification and labelling that relies on the developmental toxicity outcome of effects elicited by any dose by any route should be reconsidered and requires revision. No account is currently taken of normal handling and use and the relevance of animal experimental data to humans. Exposure considerations of humans in the real world are thus missing in the regulatory process leading in some cases to unwarranted downstream consequences.

In the third presentation, Dr. Stahlmann illustrated with specific examples that high oral bolus doses, as they are generally applied in OECD 414 studies, may only be incompletely absorbed from the gastrointestinal tract of small laboratory animals. Thus, the systemic uptake and distribution (internal exposure) becomes much lower than anticipated. But pharmacokinetic/toxicokinetic measurements are typically not performed on agrochemicals and industrial chemicals during the entire process of developmental toxicity hazard evaluations. Studies with numerous substances have provided general insights about species and sex differences in chemical metabolism. Occasionally some data have been generated regarding metabolic disposition changes brought about by pregnancy. However, it is difficult to make generalisations, and a chemical-by-chemical approach seems to be required. Furthermore, metabolism of chemicals in the liver, during first pass following gastrointestinal tract absorption, can be highly dose-dependent and profoundly affect toxicokinetics. The metabolic fate thus becomes entirely different from that following another route or mode of administration, which would much more closely mimic human exposure during normal handling and use. The presentation concluded that major efforts should be made to generate pharmacokinetic/toxicokinetic data in developmental toxicity hazard identification studies. The route and mode of administration of the test chemical should reflect the anticipated human exposure as closely as possible, so that the data would provide a basis for rational comparisons between species.

The fourth presentation by Dr. Daston addressed specifically maternal toxicity and concurrent developmental toxicity manifestations in rats. He described experimental results that have identified several potential mechanisms for maternally mediated developmental toxicity. The hypothesis has evolved that it is technically feasible to include markers for some common mechanisms into the standard testing protocols. Those factors go beyond the present simple end points of maternal feed consumption, body weight development and obvious manifestations of chemically induced toxicity (so called *"clinical signs"*). The proposal made by Dr. Daston was to incorporate new biomarkers concerning the acute phase response (e.g. acute phase proteins in serum, serum zinc concentration, or hepatic metallothionein concentration) and potentially make those components an integral part of the study design if they turn out to be meaningful once explored with more compounds. The new information that would be obtained could make a useful contribution to the unsolved question as to what level of a maternal effect is needed to concomitantly cause developmental toxicity. If expanded end points are measured as part of dose-range finding studies, these biomarkers could serve to identify a maximum tolerable dose that triggers the onset of objectively measurable signs of toxicity for a subsequent definitive developmental toxicity hazard identification study.

These four presentations set the stage for a plenary discussion on the topic "What should be the appropriate definition of maternal toxicity?" This was first discussed during the morning session, and the debate was resumed later in the day as the closing agenda item.

Breakout Group Reports

1. ***Does pregnancy preclude the use of toxicity data from other studies?***
2. ***What is the impact of mode and route of chemical administration on the manifestations of maternal and developmental toxicity?***
3. ***Do further end points improve differentiation between maternal toxicity and manifestations of developmental toxicity?***

Each participant had a free choice in selecting his/her topic for the breakout groups. This led to a roughly even distribution between the three topics. The outcome of these small group deliberations was then summarised by a group *rapporteur* in front of the plenary session for further discussion.

Report on Topic #1: Does pregnancy preclude the use of toxicity data from other studies?

Chairman: Dr. Sullivan, *Rapporteur*: Dr. Christian

The report made to the plenary session stated that a decision could only be made on a case-by-case, i.e. chemical-by-chemical, basis. At the outset the feeling may be that observations from other studies would not be applicable to the design of a pending developmental toxicity study, but many other factors should be considered. The route and mode of administration in 28 and 90-day studies are often different (e.g. dosed feed vs. gavage) and do not involve pregnant females. Therefore, it is recognised, based on established toxicokinetic and toxicodynamic grounds, reviewed in Dr. Stahlmann's presentation, that such preceding studies might provide different results from toxicity observations in pregnant animals (see also Topic 2 breakout group report, below). Furthermore, the age of the test animals is often different, yet test species and strain may be common factors. For example, there may be qualitative similarities in clinical signs between non-pregnant and pregnant animals; however, it may be difficult to demonstrate quantitative equivalence. Nevertheless, the findings from 28- and 90-day studies may give important clues for consideration of potential target tissues. That information can be included in a subsequent dose-range-finding study in pregnant animals or risk considerations. The outcome of developmental toxicity dose range finding studies with daily body weight and feed consumption monitoring, including clinical observations and signs of toxicity, should then be placed into the context of other data and supplement the information of a full study.

The observations are appropriate for use in the identification of perturbed maternal homeostasis.

Another point made by this breakout group was that the currently applied approach of EU regulatory agencies, based on hazard identification from developmental toxicity studies, is not taking into consideration exposure and human risk assessment.

Report on Topic #2: What is the impact of mode and route of chemical administration on the manifestations of maternal and developmental toxicity?

Chairman: Dr. Stahlmann, *Rapporteur* : Dr. Reuter
This group reconfirmed what the database shows so clearly, i.e. OECD 414 testing is usually done by gavage. The chemical industry has tended to use this conservative oral route for testing that is endorsed in the revised OECD 414 test guideline of 2001. However, for classification and risk assessment the relevant routes of human exposure should be taken into consideration. Among them is entry of chemicals into the mammalian body by inhalation, via the dermal route and by more protracted oral intake with feed and drink than gavage provides.

Returning to the conclusions arrived at with Topic #1, this group also emphasised that the situation is different for each chemical. Although desirable, for cost reasons comparative studies with different routes and modes of application in most cases are not feasible.

The group endorsed the message, delivered by Dr. Stahlmann's presentation, that kinetic data are needed to compare the findings of different studies with variable modes of application, e.g. dermal study toxicokinetic data. Data should be available on the areas under the curve in time vs. concentration plots (= AUC), and skin penetration and metabolism would give a good assessment. This breakout group largely identified with the concepts presented earlier in the Workshop.

The report noted the fact that at the present time there is insufficient information available about the impact of route and mode of application on manifestations of maternal toxicity. Data from a conventional OECD 414 study about a given chemical's effect on clinical chemistry and haematology should be established and compared with those from other conventional studies. Substance-specific effects and information from other studies should be used (e.g. haematology) to identify the most relevant end points and to supplement routine guideline parameters.

The group also commented specifically on some routes of administration that one might contemplate in the design of developmental hazard identification studies. They felt that on occasion subcutaneous injection might be appropriate. One can envision that this mode of administration could be useful if, for example, dermal exposure was to be anticipated in humans during normal use and handling. However, the test chemical could be a skin irritant on intact skin of rats, but was tolerated upon daily repeated depositions into the subcutaneous space. The advantage of subcutaneous injection is that it eliminates first pass liver metabolism and may lead to more complete absorption, while the disadvantage is extrapolation to the human routes of exposure. In contrast the group felt that intraperitoneal injections were inappropriate for reproductive and developmental toxicity studies.

One additional topic addressed was the need for testing in rabbits as the second species. A clear preference was voiced for good toxicokinetic measurements in the rat, for mechanistic data and information from humans over screening data from a second animal species.

Report on Topic #3: Do further end points improve differentiation between maternal toxicity and manifestations of developmental toxicity?

Chairman: Dr. Daston, *Rapporteurs*: Dr. Hellwig and Dr. Holzum

The participants of this breakout group reached consensus that added end points could help with a more precise differentiation in toxicity manifestations. There remained disagreement, however, as to whether added maternal response end points, in and of themselves, would be sufficient to alter the regulatory process for classifying agents as reproductive toxicants, assuming at present that the hazard based approach will be retained in the future. In particular, the group members based in regulatory agencies and involved in classification would like to see a high standard of proof that would include research data demonstrating the causal relationship between the added maternal end points and adverse developmental outcome. Furthermore, they would prefer to be convinced by data that there existed a (quantitative) relationship between the expanded maternal end points and adverse development. The critical question is whether the maternal response must reach a certain level of severity before it becomes developmentally adverse.

Based on the presentations in the workshop, as well as research conducted in the laboratories of those present, it was felt that the new maternal end points would most likely add value to developmental toxicity studies. Among the expanded measurements would be those that evaluate the acute phase response (e.g. acute phase proteins in serum, serum zinc concentration, or hepatic metallothionein

concentration), haematology, clinical chemistry/clinical pathology, organ weights or histopathology of selected organs. Histopathology would be limited to those organs identified as targets in 28- or 90-day sub chronic studies.

The first suggestion of this breakout group was to evaluate 5 or 6 compounds from among chemicals already tested in an OECD 414 study. These would be compounds that have caused developmental toxicity of equivocal origin (primary vs. secondary effects). Those chemicals from the "grey zone" should be examined for their acute phase response –on the selected end points mentioned above–, as a means of demonstrating the utility of those added criteria. Given the concerns of some participants in the breakout group, it would be important to supplement that information with experiments like those described in Dr. Daston's presentation in the acute phase response that is determined within hours after administration of the chemical. The new data might demonstrate the causal relationship between these end points of maternal toxicity and their association with abnormal development. Included should be a temporal correlation between the onset of maternal effects and the developmental effects, and dose-response measurements as regards the onset, magnitude and progression in intensity of maternal effects with temporal correlation and detection of an emerging threshold for abnormal development.

It is conceivable that the dose-response data from studies with expanded and more sensitive end points could in some instances lead to a narrower dosing range in future developmental toxicity studies. It is well known that dose-response curves in pharmacology and toxicology can be very steep. A 3-4 fold increase in dose between treatment groups, as is often applied in developmental toxicology, may have profound impact and may be too high if new end points are applied to characterise the perturbation of maternal homeostasis. There are very few good dose response data available on the correlation between maternal and developmental toxicity. Without more sensitive end points the question cannot be decided at present.

The second proposal made was to ask chemical companies to re-examine their toxicology databases, including developmental toxicity studies, for correlations between expanded maternal end points (e.g. clinical chemistry) and adverse developmental outcome since it is possible that such data have previously been collected. One concern was that measurements of the maternal end points conducted near term (rat gestation day 20) in the past may not be useful for those studies in which under the old test guidelines dosing ceased on gestation day 15. Some comparisons have already been done and concluded that maternal clinical chemistry effects were measurable on gestation day 20 when dosing had continued through gestation day 19; however, when dosing had ended on gestation day 15

those effects were not present. Therefore, only studies conducted with the new test guidelines may be meaningful for this retrospective analysis.

A third suggestion was to hold a second workshop to evaluate actual data sets in which expanded maternal end points had been collected. This proposal would seem reasonable if the first recommendation to evaluate 5 or 6 chemicals from the "grey zone" would be acted upon.

3. KEY DISCUSSION POINTS ON PLENARY LECTURES AND BREAKOUT GROUPS

3.1 What should be the appropriate definition of maternal toxicity?

In the final part of the Workshop the discussion of that topic was resumed in plenary session. As a take-off point for the debate the proposed definition of maternal toxicity was:

"It involves disturbance of maternal homeostasis that may affect normal development of the embryo/foetus. The latter effect is secondary to maternal toxicity without direct effects on the embryo/foetus. "

At the end of the discussions, this definition, without more elaborate qualifications, was not satisfactory for all participants of the Workshop.

The tools to differentiate between primary and secondary effects include several general parameters that have been recorded all along in guideline-compliant studies, among them body weight gain, feed consumption and clinical observations for signs of toxicity.

Among specific parameters that should be considered were:

1. Chemical-specific mechanistic information from other studies.
2. Additional measurements to evaluate maternal homeostasis, such as blood gases, haematology and key enzymatic parameters (e.g. liver enzyme induction).

One argument made was that adverse effects on the embryo/foetus are the only relevant outcome. Under the present hazard-based regulatory system of classification it does not matter whether this is a primary or secondary effect or a combination of the two.

The test guidelines encourage use of high dose studies, which depending on the toxic properties of the specific chemical may cause clear signs of parental/maternal toxicity. Even some maternal deaths are an acceptable study outcome. Changes in maternal homeostasis could be detected before overt toxicity is observed if more sensitive end points would be applied. That would meet the criterion of raising doses to maternally toxic levels.

The extrapolation of animal developmental hazard evaluation data should be made in light of potential human exposures. Only risk-based decisions would be meaningful to protect people because that is the purpose of the entire testing program.

3.2 Does pregnancy preclude the use of toxicity data from other studies?

Decisions can only be made on a chemical-by-chemical basis and many other factors should be considered. Despite differences in route and mode of administration in 28 and 90-day studies and lack of information from pregnant females, the findings from 28- and 90-day studies may give important clues for consideration of potential target tissues.

The discussions also questioned the currently applied approach of EU regulatory agencies to classification of developmentally toxic chemicals based on hazard rather than on risk.

3.3 What is the impact of mode and route of chemical administration on the manifestations of maternal and developmental toxicity?

The profound impact that mode and route of administration can have on toxicity was explicitly recognised. The route and mode of application chosen for animal hazard identification testing should mimic anticipated human exposure as closely as possible if the physical-chemical and toxicological properties of the chemical identified in preceding studies permit. Furthermore, mode of administration should be as comparable as possible with other studies.

There was a keen sense of awareness about the profound consequences of how a chemical enters into an intact mammalian organism on toxicokinetics and toxicodynamics – and the resulting biological consequences. Inappropriate means of administration of a chemical to pregnant test animals could lead to results with questionable relevance for the human population that is potentially at risk when exposed by an entirely different mode and route.

The historical database for developmental toxicity hazard evaluations of chemicals in laboratory animals is built on studies conducted mostly by daily oral bolus administrations (gavage) throughout a substantial part of pregnancy. Changes in the duration of chemical administration were introduced in 2001. In rats dosing was extended by 4 days from gestation days 6-15 to 6-19. Therefore, a majority of the studies on record were conducted with the old test guidelines. Gavage administration keeps the daily dose per unit body weight constant as pregnancy progresses into the stage of rat foetogenesis beyond gestation day 15. Only the uterine contents (i.e. foetuses) grow at a dramatic pace during the foetal phase of prenatal development. However, dosing continues based on the total body weight of the dams, including the gravid uterus.

The scientific rationale for gavage with its associated toxicokinetics and toxicodynamics is difficult to envision in the light of likely real world exposures of humans during normal handling and use of chemicals. Intake by a daily oral bolus, while still endorsed by the OECD 414 test guidelines, is not the anticipated mode of entry of chemicals into the human body. That mode of exposure in animal studies, therefore, appears to be of limited relevance to assess developmental toxicity potential in pregnant women who typically are exposed to much lower daily doses by more protracted means (food and drinking water).

The problems created by regulatory classification based on developmental toxicity hazard were brought up repeatedly during the Workshop. Regulatory agency representatives reminded all participants that the Workshop was to focus on chemically induced maternal toxicity and its relationship to developmental toxicity. Regardless, the discussion returned time and again to the problem of overestimating human developmental toxicity potential caused by animal testing hazard-based classification.

3.4 Do further end points improve differentiation between maternal toxicity and manifestations of developmental toxicity?

Workshop participants solidly supported acceptance of a recommendation that expanded end point studies might be very useful although there was no unanimous (or close to unanimous) endorsement of that concept. Some attendees remained unconvinced that refinements in detection of the maternal toxic response would help resolving the classification issue since any adverse developmental outcome would still trigger classification as long as the system is hazard based.

There was broad support for the recommendation that a search of existing databases and design of study protocols to expand maternal end points concurrent with embryo/foetus end points to assess the developmental consequences could be a useful experimental approach. The exploratory data shown by Dr. Daston suggest that the biochemical factors determined in the acute phase of maternal response, soon after administration of a test chemical, were very informative about the developmental *sequelae*.

Workshop participants who are actively involved in animal studies and experimental work solidly supported the view that selection of 5 or 6 chemicals with equivocal OECD 414 test outcomes as to the origin of adverse developmental effects could become the first step leading to exploring more sensitive end points of maternal toxicity.

However there was also reluctance to endorse examining expanded end points in order to refine the crude maternal toxicity criteria that have been applied in the past > 20 years in OECD 414 guideline compliant studies. The underlying premise is that the hazard-driven regulatory decision process towards classification and labelling will remain the operating foundation in the future. Even if a maternally mediated mode of action leading to concurrent developmental toxicity seems to occur, it is the developmental toxicity that determines classification. Thus, in the present regulatory framework human exposure conditions during normal handling and use are not given the relevance that they should be given for classification. However, there was curiosity as to how much expression of maternal toxicity might, for example, cause a 10% reduction in foetal body weights. Such information is unavailable at the present time. Systematic experiments have not been performed as to what level of maternal toxicity will produce defined changes in the embryo/foetus.

4. CONCLUSIONS AND RECOMMENDATIONS

4.1 The current regulatory classifications and their consequences for chemicals that have caused developmental toxicity in OECD 414 testing are causing concern. This is due to the lack of differentiation concerning the origin of adverse developmental effects caused through maternal toxicity (secondary effects) as opposed to those produced by direct events on the embryo/foetus (primary action). The recommendation by a majority of Workshop participants was to move to a process that considers anticipated human exposure during normal handling and use. This would introduce risk characterisation as an important component into the regulation of chemicals in commerce and trade, replacing the current hazard-identification based approach.

4.2 The relevance of route and mode of administration of test chemicals emerged as an important issue for classification of chemicals. Equally weighted were dose-response data as well as toxicokinetics and toxicodynamics, which were recognised as crucial criteria that should be considered in the study design.

The scientific evidence that the biological and toxicological effects differ profoundly depending on how the chemical enters into the body can no longer be ignored. Relevant in this context might be recent experimental comparisons in rats of gestational doses delivered by gavage vs. continuous exposure via dosed feed or drinking water. The data indicated that gavage might enhance maternal and perinatal toxicity in some studies ("Comparison of gestational dose [mg/kg] in gavage vs. continuous exposure studies in rats"; S. Parker et al, Abstract #197, Society of Toxicology 43rd Annual Meeting, 21-25 March 2004, Baltimore, MD, USA; Toxicol Sci Supplement). If gavage administration is being contemplated, then an adjustment of the daily dose is being proposed, based solely on the dams body weight proper under exclusion of the gravid uterus weight, during the last trimester of rat pregnancy to reduce confounding factors in study data interpretation.

The resulting recommendation was that dose-response as well as the rationale for choosing route and mode of administration in OECD 414 testing should be reconsidered when designing such studies.

4.3 The experimental exploration of expanded end points received endorsement from a majority of the participants. Existing databases from previously conducted studies should be scrutinised for toxicity information potentially relevant to the present broadened concerns. New data with refined end points to detect the onset of maternal toxicity might improve the interpretation of the results of such studies compared to the information collected under the present

OECD 414 test guidelines. An expanded database might provide a scientific basis for industry and regulators in decision-making leading to classification. However, complete consensus was not reached due to the reservations expressed by some of the Workshop participants. There remained doubts that such new data might be constructive as long as classification is a hazard-based process.

An experimental way forward to better define maternal and developmental toxicity in OECD 414 studies received broad support. New data with refined end points to detect the onset of maternal toxicity might improve the interpretation of the results of such studies and might provide a scientific basis for industry and regulators in decision-making leading to classification.

The recommendation was to evaluate 5 or 6 compounds from among chemicals with equivocal test outcome (primary vs. secondary effects on the embryo/foetus; the so-called "grey zone") for their effects on selected expanded maternal end points (acute phase response, haematology, clinical chemistry) as a means of demonstrating the utility of those added criteria.

4.4 Implementation of the recommendation in item 4.3 would require research funds to conduct the studies. The Long-range Research Initiative (LRI) as a continuation of this LRI–sponsored Workshop should deliberate the merits of such experiments.

The recommendation is, therefore, that Cefic/LRI should consider funding research to examine the added value of expanded end points in the characterisation of maternal toxicity.

4.5 Once the work proposed in item 4.4 is completed, another recommendation is that the overall outcome should be evaluated in a future LRI-funded workshop. The objective of that meeting would be to determine the research accomplishments.

APPENDIX 1: WORKSHOP PROGRAMME

Tuesday 2 March 2004

08.30-09.00	Registration and coffee	
09.00-09.05	**Welcome**	Dr. Mike Gribble
09.05-09.15	**Introduction**	Prof. Geoff Randall
09.15-09.45	**Background and Issues of Maternal Toxicity**	Dr. Frank Welsch
	Maternal Toxicity: Difficulties in Data Interpretation of Developmental Toxicity Studies and Their Impact on Classification and Labelling	Prof. Frank Sullivan
	Mode and Route of Administration and the Consequences for Metabolism and Kinetics	Prof. Ralf Stahlmann
	Expanded Maternal Endpoints and Their Added Value for Developmental Toxicity Study Interpretation	Dr. George Daston
11.15-11.30	Coffee Break	
11.30-12.30	Plenary Discussion: **What should be the appropriate definition of maternal toxicity?**	
12.30-13.30	Lunch	
13.30-15.00	Breakout Groups: • Does pregnancy preclude the use of toxicity data from other studies? • What is the impact of mode and route of chemical administration on the manifestations of maternal and developmental toxicity? • Do further endpoints improve differentiation between maternal toxicity and manifestations on developmental toxicity?	
	Coffee Break	
15.30-16.30	**Report and Conclusions from Breakout Groups**	
16.30-17.00	**General Conclusions and Potential Research Directions**	

APPENDIX 2: PRESENTATION ABSTRACTS

Background and Issues of Maternal Toxicity

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Developmental toxicity hazard assessments for chemicals to enter commerce and trade in the EU are presently being conducted and quality assured by regulatory agency test guidelines (e.g., OECD 414). Those guidelines were last updated and revised in 2001. An unsettled issue remains the adequate distinction between maternal toxicity caused by high doses of the test chemical, given as stipulated in the test guidelines, and concurrent developmental toxicity. The test guideline criteria defining maternal toxicity remain much cruder than those applied to prenatal development. The OECD 414 guidelines stipulate exposure of pregnant animals to high doses, and maternal toxicity is commonly elicited as a side effect. Concurrently there occur manifestations of developmental toxicity when near term foetuses are examined. The interpretation of the cause of such adverse effects on prenatal development has profound consequences for classification and labelling in commerce and trade. It is known that at times the disruption of maternal homeostasis and well being by chemically induced toxicity may secondarily affect the normal development of the embryo/foetus. However, the prenatally elicited toxicity may also be directly related to administration of the test chemical. The distinction is sometimes equivocal and causes conflicts between scientists who conduct the studies and those in regulatory agencies who are charged with protecting human health and the environment. There are noticeable shortcomings in the presently recorded end points that define maternal toxicity compared to those applied to delineate effects on the products of conception. Exploratory studies are on record that provide additional information to enhance the information provided by more typical end points of maternal toxicity. Such additional data may add sensitivity to detecting the onset of maternal toxicity. Those preliminary results may provide more precise discriminations between chemically induced maternal toxicity causing developmental toxicity indirectly and adverse effects elicited by direct action of the test substance on the embryo/foetus. The introductory presentation to this ECETOC workshop builds on the previously distributed background paper and will set the stage for discussions to review the present state of affairs. The information may lead to research recommendations that may allow less equivocal cause-and-effect associations. A need for additional data may evolve from the workshop presentations and the break-out group as well as joint discussions. Priority recommendations for the most promising research leads might emerge.

Maternal Toxicity: Difficulties in Data Interpretation of Developmental Toxicity Studies and their Impact on Classification and Labelling

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Classification of chemicals for toxicity to reproduction was introduced in the Dangerous Substances Directive 69/549/EEC in 1992. To indicate the type of chemical which should be classified the following words were used: "Classification of chemicals as toxic to reproduction is intended to be used for chemicals which have an intrinsic or specific property to produce such toxic effects. Chemicals should not be classified as toxic to reproduction where such effects are solely produced as a non-specific secondary consequence of other toxic effects. Chemicals of most concern are those which are toxic to reproduction at exposure levels which do not produce other signs of toxicity." In the past ten years since the introduction of this classification system two rather polarised views have been taken of this wording. The Classification Working Group (CMR WG) of the Commission have tended to focus on the words 'intrinsic property' and since the system is hazard based rather than risk based, have interpreted the Directive as meaning that if any dose of a chemical by any route shows reproductive toxicity, then it should be classified. The Industry scientists have focussed on the words 'exposure levels which do not produce other signs of toxicity.' Because of the serious consequences of classification on subsequent use of chemicals, this polarisation of focus has led to repeated arguments and discussions and recently even to threats of legal action.

In this presentation the initial intentions of the classification system will be discussed, along with some factors leading to the present disputes, and some remedies proposed.

Analysis of studies on reproductive toxicity both for effects on fertility and on development is complex. Primarily, reproductive toxicity follows the same rules as other types of toxicity and is subject to the same general principles as laid down by Paracelsus in the 16th Century 'All substances are poisons; there is none which is not a poison. The right dose differentiates a poison from a remedy.' In addition however, some substances have the potential to selectively affect the developing embryo to cause malformations at dose levels below those causing any other adverse effects. Special efforts should be made to identify such substances and to limit exposures of pregnant women, without at the same time classifying a large number of other chemicals, with serious economic consequences, and no benefits to public heath.

Both Industry and Regulators have tried to adopt a simple single step approach to classification for reproductive toxicity by looking at a single (or several similar) studies and making a decision based on the results. For many of the chemicals now classified the Regulators have said there is clearly evidence of reproductive toxicity therefore it should be classified, and the Industry has responded by saying there is maternal toxicity therefore it should not be classified. This has lead in the past few months to the EU CMR WG asking for a set of rules that can be applied to decision-making on whether the effects are secondary to maternal toxicity or not. In this presentation I will show why this is an impossible request and adoption of a two-step approach is necessary. The first step is a hazard assessment (identification and characterisation), and the second step is a mechanistic analysis to identify whether the observed effects would be likely to occur in humans, at exposure levels of interest.

The conduct of developmental toxicity tests used by the chemical industry will be briefly discussed along with the relevance of these with respect to classification. Different aspects of the relationship between maternal toxicity and developmental toxicity will be discussed. On the basis of mechanistic studies, chemicals can be subdivided into various subgroups: One in which the developmental toxicity is not related to maternal toxicity and the chemical should be classified; two classes where the effects are related to maternal toxicity but should not lead to classification; where the effects are seen in the presence of maternal toxicity but cannot be shown to interdependent. In the latter group, the importance of other factors will be discussed which indicate whether classification is justified or not.

The relevance of the route of administration and normal handling and use in making the decision will be discussed.

The Conclusion of the presentation will be that, considering the objectives of the classification proposals in the Dangerous Substances Directive, a weight of evidence approach is necessary. Decisions based primarily on either 'any dose by any route' or 'only in the absence of maternal toxicity' are inadequate. The mechanism of action, dose, route, class of effect as major or minor, dose-response relationships, presence of a threshold, normal handling and use, consideration of potency cut-offs have to be taken into account. All have to be considered against a background understanding of the consequences of classification.

Mode and Route of Administration and the Consequences for Metabolism and Kinetics

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Most drugs as well as other xenobiotics are given orally when tested for their toxicity. The main reason for this approach lies in the fact that human exposure - besides inhalation and perhaps dermal exposure - occurs mainly via the oral route. However, the kinetics of a chemical after its oral ingestion is influenced in many ways, which can lead to major differences in the exposure of an adult or developing organism when compared to the relevant human exposure. It is well known that high oral doses, as often applied during toxicity studies, are only incompletely absorbed from the gastrointestinal tract of small laboratory animals and that the systemic exposure is far lower than expected. In such cases the comparison of the doses applied in animals and man will lead to an underestimation of possible risks.

An interesting situation exists with the quinolone moxifloxacin, which shall be mentioned as an example for such a situation. Orally applied doses of moxifloxacin during a teratogenicity test were two orders of magnitude higher than those recommended for therapy (500 mg/kg vs. 5 mg/kg). Nevertheless, the systemic exposure in pregnant rats was considerably lower than the average human exposure (only approx. 25 % based on AUC-values). Interestingly, maternal toxicity was reported to occur under these conditions, which limit the possibility to test the compound at multiple dosing regimens. As a general rule, the following aspects must always be taken into careful consideration when data from a study on the developmental toxicity of a chemical are evaluated: maternal adsorption, distribution, protein binding in plasma and tissue, elimination via the kidney, liver or other routes, first pass effect, placental transfer, distribution and metabolism within the embryonic/foetal compartment and - possibly - aspects of lactational transfer and kinetics of the compound in offspring.

Many pharmacokinetic studies show that the kinetics and in particular the metabolism of a compound can be sex dependent and that it differs in pregnancy in comparison to the non-pregnant status as well as in the newborn of juvenile organism in comparison to an adult. Despite the same route of exposure ("orally"), major differences can be found among some compounds if the kinetics of high, toxicologically relevant doses (bolus) are compared with the kinetics of human exposure, which often occurs more protracted. The main determinant of the toxicity of a chemical may be the peak concentration (C_{max}), but also the AUC

values or the length of time above a certain threshold concentration can be crucial for toxic effects to occur.

Despite the fact that these relationships are known in principle, they are usually unclear for a chemical when the toxicity test is performed. Major efforts should be made to provide detailed pharmacokinetic data of the compounds tested for developmental toxicity in animal as well as in humans and to select a route and mode of administration which mimics the human exposure most closely to provide a basis for a rational comparison between species.

Expanded Maternal Endpoints and their Added Value for Developmental Toxicity Study Interpretation

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It is often suspected that developmental toxicity observed only in conjunction with maternal toxicity is attributable to some aspect of the maternal response; however, the maternal data from standard developmental toxicity study designs is insufficient to fully support such a conclusion. As a consequence, the more conservative assumption – that the developmental effects are the direct result of the tested chemical – is often taken to be true. As the field of developmental toxicology has progressed, we have discovered a number of potential mechanisms for maternally-mediated developmental toxicity. It is technically feasible to include markers for some of the more common mechanisms into standard protocols as a means of resolving whether the observed developmental effects are secondary to maternal toxicity.

A number of maternally-mediated mechanisms that have adverse developmental consequences have been described in the literature. Two of the more common are 1) effects leading to embryonic hypoxia, including decreased uterine blood flow, anaemia, or interference with the oxygen carrying capacity of blood; and 2) effects leading to embryonic zinc deficiency as part of a systemic acute phase response. Examples of agents that induce developmental toxicity by interfering with the mother's ability to deliver oxygen to the conceptus are 1) vasopressin or epinephrine, which restrict uterine blood flow; 2) the non-steroidal anti-inflammatory drug diflunisal, which produces haemolytic anaemia in rabbits but not other species; and 3) carbon monoxide, which forms carboxyhaemoglobin and thereby reduces the amount of oxygen that can be carried by the blood. The acute phase reaction is a generalized response to tissue injury or significant inflammation at any site in the body. It is mediated via the release of inflammatory mediators and involves numerous changes in plasma protein profiles as well as the induction of metallothionein (MT), a zinc-binding protein, in the liver. The MT induction is robust and can be sufficiently great in magnitude to evoke a systemic redistribution of zinc, temporarily decreasing the concentration of zinc in the circulation. In the pregnant animal this leaves the embryo transiently zinc deficient, a condition that is developmentally adverse. We have shown that the decreased availability of zinc to the conceptus causes abnormal development, and that a large number of diverse chemicals exert their developmental effects via this maternally-mediated mechanism.

There are a number of clinical biomarkers of the acute phase response that could be measured as part of a developmental toxicity protocol, such as circulating levels of specific inflammatory mediators, or more simply, serum zinc concentration. It is also relatively straightforward to evaluate hypoxia by measuring oxygen tension in blood and to do standard clinical haematology to detect anaemic conditions. These could be used as routine biomarkers of maternal toxicity in standard study designs, or in range-finding studies as indicators of having reached a maximum tolerable dose. In summary, we have sufficient research to make changes in study designs that will help solve the problems in interpretation posed by maternal toxicity.

APPENDIX 3: BACKGROUND PAPER ON THE STATE OF THE SCIENCE

Prepared for the ECETOC Workshop on 2 March 2004, Berlin

Maternal Toxicity and its Potential Association with Adverse Developmental Effects in Prenatal Toxicity Studies

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1. EXECUTIVE SUMMARY

Developmental toxicity hazard assessments of chemicals are conducted under regulatory test guidelines. In the EU such studies adhere to the OECD 414 recommendations that were updated in 2001. The end points by which maternal and developmental toxicity are presently assessed are reviewed here, and it appears that the criteria which define maternal toxicity remain rather crude compared to those that are applied to prenatal development. Upon exposure of pregnant animals to high doses as stipulated in OECD 414, maternal toxicity is commonly elicited. Concurrently there are often manifestations of developmental toxicity when the fetuses are examined. The interpretation of the cause of those adverse effects on prenatal development has profound consequences for classification and labeling in commerce and trade. Perturbation of maternal well being by chemically induced toxicity may secondarily affect the normal development of the embryo/fetus. On the other hand, the prenatally elicited toxicity may be directly related to administration of the test chemical. It is this sometimes equivocal distinction that causes differences of opinions between the scientists who conduct the studies and those in regulatory agencies charged with protecting human health and the environment. The shortcomings of the presently recorded end points defining maternal toxicity are described in this paper. The outcome of exploratory studies that provide additional information and may add sensitivity to detecting the onset of maternal toxicity is also considered. The results may offer the possibility of allowing more precise discriminations between chemically induced maternal toxicity with indirectly associated developmental toxicity, or effects caused by direct action of the substance on the embryo/fetus. This workshop, organized by ECETOC, will bring together scientists from academia, industry and regulatory agencies to review the information and make recommendations that would allow less equivocal cause-and-effect associations. Specific research needs may emerge from the workshop discussions with priority recommendations for the most promising leads.

2. INTRODUCTION AND AIMS OF THIS PAPER IN PREPARATION OF A TOPICAL WORKSHOP

Developmental toxicity hazard assessment studies of industrial chemicals and agrochemicals are performed in compliance with regulatory agency test guidelines, such as OECD 414. In the past differences of opinion have sometimes arisen in the interpretation of animal test data when a test article, given at high doses as stipulated in the guidelines, elicits maternal toxicity. Toxicity manifestations in pregnant laboratory animals often occur concurrently with developmental toxicity that is detected in near term fetuses.

Developmental toxicity is one of the important end points in the classification, labeling and regulation of chemical agents for commerce and trade. Regulatory agencies also include maternal toxicity as a serious criterion in the classification and labeling decisions. Therefore differences in data interpretation about possible cause-and-effect associations between maternal toxicity in conjunction with developmental consequences may create conflicting opinions. Among the underlying and significantly contributing factors may be the test end points that define maternal toxicity. Lack of sufficient knowledge about how perturbation of maternal physiological homeostasis might indirectly affect development of the embryo/fetus may lead to classification and labeling of an agent as a developmental toxicant when, in fact, the apparent adverse outcome caused by chemical treatment of the dam may have its origins in the maternal organism.

The objective of this document is to provide background information about the present approach to developmental toxicity hazard assessments. Furthermore, the paper serves to illustrate what is known about the impact of a chemically induced maternal response on embryonal/fetal development. The aim is to set the stage for a workshop that will bring together scientists to address issues of maternal and associated developmental toxicity. The charge is to review the necessary information for appropriate classification and labeling. In addition, if so indicated by the workshop discussions, directions for potential research may emerge that would be expected to provide more complete information.

2.1 Present Status of Prenatal Developmental Toxicity Testing

2.1.1 Test guidelines and recent revisions

Soon after governmental regulatory agency test guidelines to assess developmental toxicity hazard potential of chemicals were implemented, it became apparent that the stipulated high dose treatments of pregnant laboratory animals might

confound data interpretation. High exposure doses are recommended in the guidelines, with the underlying premise that high doses will enhance the chances of unmasking developmental hazard potential when the number of test animals is small. At least one of the typically used three dose levels should be chosen with the aim to induce some developmental and/or maternal toxicity (such as clinical signs or a decreased body weight; OECD 2001a; b). Among the 2001 test guideline revisions were changes over the OECD test guidelines of 1981 related to maternal toxicity. The previous version referred only to maternal toxicity and noted that the test chemical should ideally induce signs of toxicity, such as a 10% body weight loss. In some instances, the prenatally elicited embryo-/fetotoxicity may be a consequence of maternal toxicity. In other cases the developmental toxicity is caused by the intrinsic properties of the test chemical on the conceptus, an outcome that leads to the applicable classification and labeling. However, even if a distinction cannot be made where the origins of the developmental toxicity are (i.e. maternal toxicity with indirect consequences on the embryo/fetus vs. direct embryo/fetus effects), the observed effects may be considered independent of the concurrent maternal toxicity. The test chemical is then classified and labeled accordingly by regulatory agencies.

The present document leans on the test guidelines provided by OECD 414 (OECD 2001a), with emphasis on prenatally induced toxicity and its manifestations in near term fetuses. The focus will be on studies conducted in rats. The reasoning behind the developmental phase restrictions and the species preference will be developed below.

The revised regulatory agency developmental toxicity test guidelines, issued by the US-EPA in 1998 (EPA 1998a; 1998b) and the OECD in 2001(OECD 2001a; 2001b), have substantially prolonged the chemical exposure duration in rats from 10 to 14 consecutive days of pregnancy. This extension has eliminated the possibility for maternal recovery in late gestation, once the 10-day treatment phase (gestation days 6-15; old guidelines) is over. The extended test article administration time (gestation days 6-19, new guidelines) has enhanced the potential to detect effects on reproductive organ differentiation that is underway during fetogenesis. Data on the correlation between chemical-induced maternal effects and dose delivered to the embryo are not required in test guideline compliant studies.

Guidance on how to evaluate reproductive toxicity data has recently been comprehensively assembled in a monograph prepared by an ECETOC Task Force (2002). The issue of maternal/parental toxicity and its potential association with developmental toxicity is actively being considered by several panels, working under the auspices of the European Chemicals Bureau. For example, ECBI/56/02 Add. 9, addresses that topic in some detail as does ECBI/33/02 in section 13.12.7.1.

Furthermore, improved guidance to help with the classification of substances toxic to reproduction is pursued in ECBI/56/02 Add. 20.

The current EU guidelines for classification and labeling address how the occurrence of maternal toxicity will influence the evaluation and affect classification and labeling by regulatory agencies (Offic. J. Europ. Comm., 2001).

2.1.2 End points of toxicity evaluations

The developmental toxicity testing guidelines of the EPA (1998 a) and OECD (2001a) are heavily focused on manifestations of developmental outcome. In contrast, the recorded maternal parameters remain rather crude. The end points of maternal effects evaluated are much less specific (Table 1) than those concerning developmental toxicity (Table 2). Body weight and weight gain during pregnancy, food consumption, grossly visible signs of toxicity (recorded as "clinical signs"), and a gross morphology/pathology inspection of the maternal carcass and major organs near term complete the maternal evaluations (Table 1).

Table 1. Endpoints of maternal toxicity *

Mortality

Mating index [(no. with seminal plugs or sperm/no. mated) × 100]

Fertility index [(no. with implants/no. of matings) × 100]

Gestation length (useful when animals are allowed to deliver pups)

Body weight

- Day 0
- During gestation
- Day of necropsy

Body weight change

- Throughout gestation
- During treatment (including increments of time within treatment period)
- Post-treatment to sacrifice
- Corrected maternal (body weight change throughout gestation minus gravid uterine weight or litter weight at sacrifice)

Organ weights (in cases of suspected target organ toxicity, especially when supported by adverse histopathology findings)

- Absolute
- Relative to body weight
- Relative to brain weight

Food and water consumption (where relevant)

Clinical evaluations

- Types, incidence, degree, and duration of clinical signs
- Enzyme markers
- Clinical chemistries

Gross necropsy and histopathology

* Source: EPA, 1991

The end points "Enzyme markers" and "Clinical chemistries" listed in Table 1 are not a mandatory part of the data to be provided under the revised test guidelines (e.g., OECD 414; 2001). However, the value of such information has been recognized, and appropriate measurements, amongst others, are recommended in the ECETOC monograph (2002). Such studies have been performed on a case-by-case basis. A very recent Technical Guidance Document on Risk Assessment (European Commission, 2003) advocates "ideally, toxicokinetic and metabolism data should form part of the basis for a decision on the route of exposure used in the tests for reproductive toxicity." Such information is not required for industrial chemicals and commonly does not exist.

The end points recorded regarding developmental toxicity are shown in the overview of Table 2

*Table 2. Endpoints of developmental toxicity **

Litters with implants
- No. implantation sites/dam
- No. corpora lutea (CL)/dam[a]
- Percent preimplantation loss

$$\frac{(\text{CL} - \text{implantations}) \times 100}{\text{CL}}$$

- No. and percent live offspring[b]/litter
- No. and percent resorptions/litter
- No. and percent litters with resorptions
- No. and percent late fetal deaths/litter
- No. and percent nonlive (late fetal deaths + resorptions) implants/litter
- No. and percent litters with nonlive implants
- No. and percent affected (nonlive + malformed) implants/litter
- No. and percent litters with affected implants
- No. and percent litters with total resorptions
- No. and percent stillbirths/litter
- No. and percent litters with live offspring

Litters with live offspring
- No. and percent live offspring/litter
- Viability of offspring[c]
- Sex ratio/litter
- Mean offspring body weight/litter [c]
- Mean male or female body weight/litter [c]
- No. and percent offspring with external, visceral, or skeletal malformations/litter
- No. and percent malformed offspring/litter
- No. and percent litters with malformed offspring
- No. and percent malformed males or females/litter
- No. and percent offspring with external, visceral, or skeletal variations/litter
- No. and percent offspring with variations/litter
- No. and percent litters having offspring with variations
- Types and incidence of individual malformations
- Types and incidence of individual variations
- Individual offspring and their malformations and variations (grouped according to litter and dose)
- Clinical signs (type, incidence, duration, and degree)
- Gross necropsy and histopathology

[a]-Important when treatment begins prior to implantation. May be difficult to assess in mice.

[b]-Offspring refers both to fetuses observed prior to term and to pups following birth. The endpoints examined depend on the protocol used for each study.

[c]-Measured at selected intervals until termination of the study.

*Source: EPA, 1991

There is the possibility that data from 28-day (OECD, 1995) and 90-day studies conducted in rats (OECD, 1998) are already available when a developmental toxicity testing program is being scheduled. Those observations may have provided morphological indications that a chemical might pose a reproductive hazard and may provide clues as to potential target tissues or organs in which "maternal toxicity" might manifest itself at certain dose levels. Unfortunately, the route and mode of exposure in such preceding toxicity studies in rats is often different (e.g., dosed feed) from the one to be used in the prenatal developmental toxicity studies (most commonly gavage; see 2.1.4 and 2.1.5 for more details). Furthermore, it is likely that non-pregnant females were used in the 28-day and 90-day studies. The toxicity response of pregnant rats may be different.

2.1.3 Test animal species selection

The revised test guidelines (EPA 1998a; b; OECD 2001a; b) maintain the long held preference that rats and rabbits should be used as the test animal species. There is a two species mandate, applicable to developmental toxicity hazard evaluations of agrochemicals, that does not apply to industrial chemicals. The adherence to rabbits may have its foundations in the very early 1960s when the thalidomide drug disaster profoundly changed the concepts of invulnerability of the embryo/fetus to chemical insult. As a systematic testing strategy evolved in subsequent years, rabbits gained a firm foothold in the emerging regulatory test guidelines for prenatal developmental toxicity hazard assessments. The reason for this so far unquestioned reliance on rabbits may be that pregnant does of some rabbit strains responded to thalidomide with limb malformations. However, other rabbit strains were not affected by thalidomide in the same way. Induction of limb lesions in the sensitive rabbit strain required much higher doses than those estimated having caused the human embryopathies when pregnant women took thalidomide. Nevertheless, there was a positive malformation response in rabbits that did not occur in pregnant rats.

There is discussion ongoing about the scientific merits of using two species. The German VCI has an evaluation underway to review rabbit developmental toxicity data. Following many years of prenatal developmental toxicity hazard assessment experience, a very recent data review from veterinary medical therapeutic agents has been published. That overview reveals that in two species studies rabbit data enhanced the overall value of the developmental toxicity information obtained (Hurtt et al., 2003).

Over the decades during which developmental hazard assessments have been conducted under changing regulatory guidelines, rats have become the preferred species in one or two generation reproduction studies and in neurodevelopmental toxicity testing. The database for all physiological and biochemical characteristics in various strains of rats is much larger than that established for rabbits. The genetics of rats used in testing are much better known, more standardized and constantly monitored than those in rabbits. Among rodents, knowledge of genetics among all species is most advanced in mice. Much of the breakthrough work in normal developmental biology and birth defects research, both of genetic origin and chemically-induced, has derived from mice.

The second species issue deserves mentioning in the context of this background document for completeness sake. Rat studies and their interpretation are to be the focal point of the workshop.

2.1.4 Route and mode of administration of test chemicals

Both the OECD 414 test guidelines and the more recent Technical Guidance Document on Risk Assessment (European Commission, 2003) state that the test chemical usually should be given by oral intubation. This refers to dosing by daily oral bolus administration (gavage). Most of the large developmental toxicity hazard assessment database is built on this mode of entry of chemicals into the animal's body. The 2001 OECD test guidelines also mention, in item 14., that information on metabolism and toxicokinetics of the test substance should be taken into account in the study design. The example of the lifetime cancer bioassay of chloroform can serve to illustrate the importance of mode of entry of the same total daily dose per unit body weight of that chemical. A comparison of chloroform gavage vs. more protracted intake via drinking gave entirely different results where gavage induced tumors while there was no such response in the drinking water study. Similar differences may occur in developmental toxicity studies.

For the purposes of the ECETOC workshop, the above considerations are not immediately applicable. The issue of mode of administration in the design of developmental toxicity studies, with the highest relevance to anticipated human exposures, may deserve future attention. With respect to humans, gradual and long term exposures and its potential effects are important for health risk assessments.

2.1.5 Pharmaco [Toxico] kinetics (PK) and pharmaco [toxico] dynamics (PD)

The PK properties in the systemic circulation and PK/PD at potential target sites are rarely available either for the design or the interpretation of study data of chemicals destined for agricultural or industrial applications. Information about administered dose (external dose) and dose delivered to a target tissue of concern (internal dose) are not required. Even if on occasion these parameters are measured as part of an expanded study, the data typically address only one time point in gestation.

Available information indicates that pregnancy may alter the PK/PD characteristics of chemicals. In addition, it appears highly likely that there will be differences from chemical to chemical. That knowledge affects the applicability of toxicity information from other studies in non-pregnant animals. Furthermore, the administered dose with resultant maternal PK and transplacentally delivered dose are likely to change between the first day of test article administration on gestation day 6 and the last exposure 14 days later on gestation day 19.

Daily treatment with a chemical via oral bolus dosing, as compared to dietary or drinking water intake, may be expected to elicit different maternal responses. The mode of entry via the same route (i.e., oral exposure) must be anticipated to have profound effects on PK/PD. Thus the same dose (mg of chemical / kg body weight/day) is likely to cause very different physiological responses in the pregnant dams. These insights make selection of the most appropriate mode of entry of a chemical in guideline-compliant developmental hazard evaluation studies an important consideration for future discussions to optimize study design.

3. INTERPRETATION OF HIGH DOSE DATA

3.1 Time Perspective

The debate has been ongoing for a long time whether maternal toxicity elicited by high doses could indirectly cause adverse developmental effects. In the EU regulatory process that differentiation affects classification and labeling profoundly. A first comprehensive discussion of potential cause-and-effect associations between chemically induced maternal toxicity and concurrent prenatally inflicted developmental toxicity occurred in 1986. Among the major conclusions of that workshop organized by the US-EPA was that a developmental toxicity hazard assessment became necessary whenever deleterious effects on the embryo/fetus were observed, regardless of the apparent presence or absence of maternal toxicity (Kimmel et al., 1987).

Human exposure levels are typically unknown, yet anticipated to be much lower than those applied in animal testing. The goal of prenatal chemical exposure hazard assessments and dose-response evaluations is to extrapolate laboratory animal data to potential exposure levels among women of childbearing age in occupational or environmental settings.

3.2 Uncertainties in Developmental Toxicity Data Interpretation

A high degree of certainty is desired in data evaluations entering into classification and labeling for commerce and trade. Therefore, both investigators in the chemical industry and scientists in regulatory agencies want to, and need to, know whether the deliberately high dose animal testing conditions during prenatal development induce detectable biological effects in dams and act directly or indirectly on the offspring. If so it is most important for data interpretation to differentiate whether any effects revealing maternal toxicity of the test chemical and concurrent developmental toxicity in the near term fetus might be attributable to maternally mediated toxicity. Recent revisions that created the present test guidelines for prenatal toxicity hazard assessments have retained the previous criteria for characterization of “maternal toxicity” unchanged.

4. PAST APPROACHES TO PROBE ASSOCIATIONS BETWEEN MATERNAL AND DEVELOPMENTAL TOXICITY

4.1. Chronological Perspective

The first comprehensive literature survey of almost 400 studies available in the public domain and conducted in several species (Khera, 1985) noted that at high treatment levels maternal toxicity occurred in ~75 % of those studies. The tentative conclusion was reached that maternal toxicity may be regarded as an etiologic factor for embryo/fetal malformations and mortality of the products of conception. The fundamental premise that maternally mediated effects on intrauterine development exist has been accepted, but the concept that there is a consistent relationship between maternal and developmental toxicity has been criticized and is not unanimously accepted. Such differences of opinion cause conflict when they impact on classification and labeling decisions by regulatory agencies.

4.2 Experimental Approaches

The interpretation that was advanced by Khera based on studies published in the public domain has since undergone extensive debate (e.g., Kimmel et al., 1987) and some experimental evaluations.

4.2.1 Effects of diverse chemicals at maternally toxic doses

Studies were specifically designed to test the chemically induced maternal toxicity cause-and- developmental toxicity- effect linkage hypothesis. The data obtained indicate that generalizations cannot be made. Case-by-case (i.e., chemical-by-chemical) evaluations are needed (e.g., Chernoff et al., 1990). Most of the studies have been performed in mice. However, rats and rabbits are the more commonly used test species and have received less experimental attention.

4.2.2 Effects of stress

Of particular interest has been how various means of exerting "stress" (e.g., via high dose chemical treatment or physical restraint manipulations or noise) would affect the dam and her developing offspring. In the broadest sense "stress" comprises any change in homeostasis of the organism caused by the environment. Noise stress inflicted upon pregnant rats throughout gestation reduces litter size and increases the incidence of malformations (Chernoff et al., 1989). Supernumerary ribs and delays of ossification in rodent offspring are among the common end points related to chemical exposure stress (Beyer and Chernoff, 1986; Wickramaratne, 1988; Chernoff et al., 1991) or restraint-induced stress in mice (Beyer and Chernoff, 1986).

4.2.3 Alterations of body temperature

Another factor that was experimentally evaluated is body temperature. The experimental evidence is strong that hyperthermia in rats causes neural tube malformations (Carney, 1997). The human clinical observations also indicate defects of the central nervous system associated with hyperthermia. The problem is that severe disease conditions causing the febrile condition are a serious confounding factor. The experimental data are more equivocal with respect to hypothermia (Carney, 1997).

4.2.4 Manipulations of food intake

Food consumption, body weight and its pregnancy associated gain are among the routinely measured end points in developmental toxicity studies. All three are often simultaneously reduced by high dose chemical exposure. Therefore, food deprivation experiments, sometimes to the point of severe starvation, have been used in pregnant rodents and rabbits to study the consequences on embryonic/fetal development. Among the objectives was to "mimic" the weight reductions caused by chemicals in high dose hazard identification experiments. The perturbations induced by a xenobiotic chemical are likely to cause additional biological sequelae. The simple determinations of food intake, body weight and general appearance ("clinical signs") may be too superficial. Such crude measures of maternal response, indicative of exposure-induced toxicity, are likely to become detectable only at dose levels well above those that already cause adaptive and/or pathophysiological changes at lower doses.

4.2.5 Source of new data

It is noteworthy that several of the studies described were conducted in a laboratory that is part of the US-EPA. Other investigations, performed in university-based research institutes, were conducted with EPA cooperative research funding agreements. Thus the US regulatory agency deemed it important, soon after the 1987 workshop, to provide funds for this important topic of applied research. EPA felt that issues related to maternal toxicity and prenatal developmental toxicity hazard assessments fell into the purview of its mission (see further comments in section 4.3.1).

4.3 Perspective on the Maternal-Developmental Toxicity Association

The interest in resolving the uncertainties has been long-standing. This is witnessed by the 1987 EPA workshop, numerous journal review articles, and several overview chapters in three books (see Daston in Developmental Toxicology, 1994; Carney in Handbook of Experimental Pharmacology-Drug Toxicity and Embryonic Development, 1997; Hood and Miller in Handbook of Developmental Toxicology, 1996, and its revised edition, in press 2004). As regards the latter source, it is noteworthy that there are only a few new references in the updated chapter on "Maternally Mediated Effects upon Development" (Hood and Miller, in press 2004). This fact illustrates that only lip service has been paid to the claims of how important it was to elucidate the potential relationship between chemical-induced maternal toxicity and its firm linkage, if any, to concurrent developmental toxicity. Thus, the information void remains that, if filled, could provide critical data on maternal toxicity and facilitate more unequivocal classification and labeling decisions by regulatory agencies.

4.3.1 Ongoing new research

The funding history for "applied research" on the potential association between maternal and developmental toxicity from public monies, administered by governmental agencies in either Europe or the US, is not encouraging. Peer review funding priorities in the past >10 years have been heavily favoring molecular and genetic research approaches towards elucidating mechanisms of developmental toxicity. This has happened to the exclusion of other important topics. Under the prevailing priority review and financial realities only parties with a vested interest in advancing knowledge in the field of "applied research" focused on maternal-developmental toxicity (i.e., the chemical/pharmaceutical industry and regulatory agencies) can provide the support needed to conduct the necessary research.

In light of those facts, the American Chemistry Council (ACC) in the USA has recently recognized the funding needs for such studies in their LRI programs (ACC-LRI; www.uslri.org). Two Requests for Proposals (RfPs) in 2002 have solicited research proposals to address "The Impact of Maternal Toxicity in Developmental Neurotoxicity Studies" and "The Impact of Maternal Toxicity on Development of the Nervous, Reproductive, Endocrine and Immune Systems." Proposals for the latter were due in January 2003, included objectives that were much broader than the neurotoxicity focused one and covered the whole spectrum of the complex set of problems. The breadth of the research issues in that 2003 RfP of the ACC is congruent with the definition of developmental toxicity in its widest sense in section 4.2.3.3. of the Commission Directive 21001/59/EC (Offic. J. Europ. Comm., 2001).

The above comments provide some background and identify the reasons why relatively little research has been done to date. The multiple research directions, recommended in review articles and authoritative book chapters over the past ~15 years therefore, remain as viable as when they were first published. While the planned ECETOC workshop should briefly address the breadth of the problems regarding the interpretation of reproductive/developmental toxicity hazard identification tests, it would appear most productive if a priori consensus on a limited set of topics related to the OECD 414 test guidelines would be accepted as the starting premise. The selections should be made relying on the expertise of investigators and regulators. The issues of highest interest that emerge from the workshop can then be included into a RfP for work to be proposed for LRI funding through CEFIC.

From any promising research directions that may emerge from the ECETOC workshop, CEFIC should select issues for a RfP that hold the promise to be manageable in a focused research program. There is a finite budget to achieve these goals. The objective is that the new information to be generated should make a difference in the database considered in classification and labeling in the EU.

4.3.2 Chemicals with hormone-like action

The topic of endocrine system perturbation and possible associated hormonal imbalances upon prenatal and perinatal exposure is a most actively debated one in all major industrialized countries of Asia, Europe and North America. Vast numbers of chemicals are suspected as being potentially associated with adverse effects on reproductive and non-reproductive organ differentiation and function throughout life. This extremely complex topic has received close scrutiny in the "endocrine disrupters" initiatives in various countries and is on the verge of becoming an entirely new screening and testing activity with substantial funding. This is an area with complex mechanistic intricacies. Therefore, the deliberations by ECETOC and the research planning priorities for purposes of the planned workshop should exclude "endocrine disrupters."

5. POTENTIAL IMPROVEMENTS TO COLLECT MATERNAL TOXICITY DATA

5.1 Better Detection Methods to Define Dose-Response

Measurements addressing maternal physiological and biochemical end points in response to treatment with a given test chemical are not a required part of the study design under which developmental hazard assessment studies are being performed. If such information were procured, the data might describe much more precisely and objectively the maternal health status upon exposure to a chemical. One may then see a dose-response pattern emerge that might reveal what effects on the homeostatic compensation of the maternal organism would first become detectable. Furthermore, affected end points may show a dose-related intensity of effects. Additional organ systems might become affected in the overall response manifestations to ever increasing doses, and toxicity manifestations would become more intense.

Pharmacological/toxicological action of the test agent may cause dietary deficiencies that become serious confounders. With a substantially enlarged set of end points designed to better capture perturbation of maternal homeostasis, investigators would be able to define objective criteria of what constitutes initial maternal response to treatment with a chemical agent and how responses progress in intensity and complexity.

More refined experiments regarding graded maternal dose-response-relationships have not been pursued by appropriate experiments in any deliberate fashion. Such measurements might lead from subtle effects undetectable to the naked eye to obvious impairments that are among the responses in the present test guideline end point criteria associated with high doses (using the criterion of "clinical signs"). When using the present tests in pregnant laboratory animals and interpreting their outcome in dams and offspring, there is the possibility of misinterpretation of the test results obtained at maternally toxic doses. One must keep in mind that human exposures with adverse developmental consequences will sometimes also occur at maternally toxic doses, as witnessed by maternal ethanol and cocaine abuse in pregnant women. While it is generally acknowledged that in laboratory animal species maternally mediated adverse effects on their offspring occur, there is need for more refined information that will allow appropriate classification and labeling. Ultimately, the goal is to extrapolate from animal testing how the outcome might relate to human development.

5.2 Toxicity Data from Other Studies

In spite of mode of administration differences and non-pregnant vs. pregnant rats, data obtained in preceding toxicology studies (e.g., 28- or 90-day exposure data) may provide valuable clues as to potential target tissues or organs. Such information may indicate modes of action and provide clues as to how "maternal toxicity" might manifest itself at certain dose levels in OECD 414 evaluations. The insights gained from such measurements would enable investigators to define much more accurately the overall maternal health status in response to daily repeated chemical treatment. Among the adverse effects may be reduced food consumption or embryolethality, both of which could cause reduced maternal weight gain. The overall consequence, recorded as the simplistic end point "pregnancy-related weight gain," would be the same in chemical-treatment induced weight reductions as opposed to food deprivation (e.g., pair-fed controls) yet the biochemical correlates associated with chemical exposure might be quite different. Only specific biochemical response measurements of end points in the chain of events causing body weight reductions would be able to differentiate the sequelae of food restrictions on the initial phase of failure to gain weight from those occurring concurrently with exposure to a chemical agent.

One example is the investigations regarding the acute-phase hepatic response and the Zn status of pregnant rats in response to alpha-hederin. Metallothionein synthesis and plasma Zn levels in dams and embryos were examined (Daston et al., 1994). A comprehensive set of data collected supported the hypothesis that systemic changes in Zn status of the dam were caused by a hepatic acute phase response that included a marked induction of metallothionein and might be the mechanism for maternally mediated abnormal development.

There are other research data that have applied expanded end points with specific agents (e.g, Gornial et al. 1999). Clinical chemistry methods that apply automated analyzers are available (e.g., hematology; liver functions, including drug metabolizing enzymes; renal function; blood gases as markers of acid-base balance). The investment into an additional set of end points that might be targets of chemical toxicity would be offset by the benefits of more precise data. That information is to be applied for better distinctions between maternal toxicity and developmental toxicity. This would be the anticipated benefit in the classification and labeling for commerce and trade.

A broader spectrum of measurements may prove to be sensitive (e.g., clinical chemistry; expanded hematology and the hematopoetic system; micromorphology of major organs involved in the metabolism and excretion chemicals, such as liver and kidney; prototypical liver enzymes involved in xenobiotic metabolism and

their response to ever increasing doses of the test chemical; cardiovascular system and placental perfusion and function). Dose-response and PK/PD upon chemical exposure by a relevant route and mode of entry into the maternal organism and the conceptus are critical parameters that determine the biological/toxicological consequences.

5.3 Conclusions from Previously Conducted Studies

1) More sensitive end points of acute phase dose-response on the way to frank toxicity are presently not being assessed. That kind of information could define how the onset of detectable physiological changes in the mother might be related to beginning manifestations of developmental toxicity.
2) Acute maternal responses may remain undetected because of the present methods and end points by which the pregnant test animals are examined to define maternal toxicity.
3) A few past investigations provide some guidance regarding potential directions for new research. Critical information gaps may be filled that could reduce uncertainties about maternal toxicity and its potential linkage to concurrent developmental toxicity.
4) There is a wide selection of end points that might be relevant. It is essential to evaluate working hypotheses carefully and to select research topics that are plausible based on the weight of the existing scientific evidence and have a good likelihood to advance the field.
5) Better concordance in study design (e.g., dose feed vs. gavage) may enhance the utility of the 28-day and 90-day data for subsequent developmental toxicity hazard assessment studies.
6) Consideration of the most relevant route and mode of chemical exposure in future discussions of study design would enhance the value of toxicity and hazard information for extrapolations to likely human exposure scenarios.

6. CONSEQUENCES FOR FUTURE INVESTIGATIONS

6.1 Lack of Understanding "Maternal Toxicity"

A better characterization of dose response relationships with expanded end points may be needed to define the maternal organism's reactions to treatment at critical stages of embryonal/foetal development. Initially new information has to be procured on a case-by-case approach, i.e. chemical-by-chemical. As the experimental database grows, more leeway may become possible based on structure-activity-relationships, which might allow generalizations within a chemical class. The medium term goal (3-4 years away) is to fill data gaps in rats as an appropriate test species. An expanded set of refined and objective criteria regarding maternal responses is expected to emerge.

6.2 Potential Benefits of Additional Data

Without new experimental studies one cannot predict which additional end points might be valuable to reduce uncertainties in data interpretation. It will be most helpful in the planned workshop to obtain input from study directors who may have probed end points extending beyond the OECD 414 guidelines.

Additional studies may combine in vivo effects on maternal toxicity and developmental consequences with in vitro as well as ex vivo determinations. For example, the studies conducted by Daston et al. (1994) have combined animal experiments in pregnant rats with whole embryo culture. Additional animals may be needed to obtain the desired information because of the invasive nature of the required sample collections. However, this disadvantage is offset by more complete information available for classification and labeling of potential developmental toxicants.

6.3 Overall Aims of the Planned Workshop

The objective is to discuss whether additional information is needed to better understand the impact of maternal toxicity on the developing embryo/fetus for the interpretation of OECD 414 study data. If a consensus in the affirmative would be reached regarding new end points that may provide added value, experimental studies may be recommended to test the most promising hypotheses. The workshop should bring together investigators who are conducting OECD guideline studies, scientists who have explored mode of chemical action experiments in pregnant animals and scientists from the EU regulatory agencies.

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