CHEMICAL INDUSTRY'S RESEARCH PROGRAM ON HORMONALLY ACTIVE AGENTS (HAAs)

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ABSTRACT

Scientists from industry, regulatory, and academic institutions around the world are pursuing many of the questions raised by the endocrine disruption hypothesis. Continued research is needed to broaden our understanding of the relationship between hormonally active agents (HAAs) in the environment and the health and well being of humans and wildlife. The International Council of Chemical Associations has established a research program to better understand the potential health and environmental risks of chemicals. The American Chemistry Council (ACC), which represents chemical manufacturers in the United States, sponsors the largest portion of this global program. Through this program, the ACC provides funding for research performed by several academic and federal research institutions, the CIIT Centers for Health Research, and a joint research grant program with the U.S. National Institute of Environmental Health Sciences. The ACC research program is focused on two areas: (1) development, standardization, and validation of endocrine screens and tests and (2) mechanisms of action research on HAAs and developmental and reproductive biology.

INTRODUCTION

This paper presents the scope of the chemical industry research program on hormonally active agents (HAA). The industry around the world performs chemical-specific testing on their own products using standard protocols; in fact, this International Symposium on Environmental Endocrine Disrupters-2001 contains several presentations about the protocols that are under development to evaluate the impacts of agents on the endocrine system. This paper focuses on the HAA-related research activities of the chemical industry, not the standardized testing efforts.

The goal of the industry's research program is to broaden understanding of the potential relationships between HAAs in the environment and the health and well being of humans and wildlife. Much of the work is being done under the auspices of the International Council of Chemical Associations (ICCA). Three chemical associations within the ICCA have developed a very substantial research program, called the Long-Range Research Initiative (LRI). They are: the American Chemistry Council (ACC), the Japan Chemical Industry Association (JCIA), and the European Chemical Industry Council (CEFIC). The German Chemical Association (VCI) and others, as well as some individual chemical manufacturers, contribute to the research program, not just to the testing. A compilation of chemical industry-sponsored endocrine-related research is now available on the web (www.endocrinescience.org). It has descriptions of hundreds of projects and identifies publications and sources of more information.

This paper focuses on the programs of the ACC, specifically those related to (1) development, standardization, and validation of endocrine screens and tests and (2) mechanisms of action research on HAAs and developmental and reproductive biology.

DEVELOPMENT, STANDARDIZATION, AND VALIDATION OF ENDOCRINE SCREENS AND TESTS

As a routine component of product safety programs, industry around the world performs chemical-specific testing using standard protocols. Current toxicology testing protocols provide much information about potential endocrine toxicity. As noted by the Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC), for example, the current protocol for the mammalian multiple generation reproduction study contains numerous endocrine-responsive endpoints. However, even these internationally harmonized protocols may be enhanced by adding new endpoints that are more specific to the endocrine system, if such endpoints can be properly standardized, validated, and do not interfere with assessments of endpoints that are currently required.

One component of the HAA research sponsored by ACC focuses on developing improved toxicity evaluation methods and new techniques for prioritizing, screening, and testing substances for potential endocrine activity. ACC is working along with the U.S. Environmental Protection Agency (EPA) and the Organization for Economic Cooperation and Development (OECD) to develop and validate internationally standardized methods for use in a hierarchical, tiered "screening" and "testing" approach.

As Dr. Tong discussed in his presentation at this Symposium, for over 5 years now, ACC has been participating, through a Cooperative Research and Development Agreement with the U.S. Food and Drug Administration's National Center for Toxicological Research to develop and validate quantitative structure-activity relationship (QSAR) models to predict chemical binding to hormone receptors. Their overall goal is to employ such models as a component of science-based priority setting tools to be used to distinguish those substances that warrant further evaluation based on predicted hormone receptor binding affinity from those that are predicted to have negligible receptor binding affinity.

The ACC is also working with the OECD Task Force on Endocrine Disruptor Testing and Assessment as they work to standardize and validate uniform international methods for routine product safety testing. Initial efforts have focused on the uterotrophic-screening assay and the Hershberger screening assay. Dr. Timm discussed the OECD activities in his presentation at the Symposium. The uterotrophic assay should be useful to screen for estrogenic agonist and antagonist activities of agents; the Hershberger assay is intended to screen for androgenic agonist and antagonist activities.

The ACC has also sponsored research to develop new and improved screening methods. The Intact Male assay is the result of over eleven years of work by scientists within DuPont's Haskell Laboratory to develop short-duration in vivo screening methods to identify endocrine modes of action. The Intact Male assay is a short-term, sensitive and specific 'multi-modal' mechanistic assay that has been demonstrated to be capable of identifying substances that modulate the endocrine system, including compounds that have the potential to act as: (1) agonists or antagonists to estrogen, androgen, progesterone, or dopamine receptors, (2) 5alpha-reductase inhibitors, (3) steroid biosynthesis inhibitors (aromatase and testosterone biosynthesis), and (4) compounds that alter thyroid function. Recently, this assay was included by OECD in the framework of methods considered relevant to screening substances for potential endocrine activity. The Intact Male assay can identify a broad spectrum of HAAs and provides information on the mode of action of a compound.

MECHANISMS OF ACTION OF HAAs AND DEVELOPMENTAL AND REPRODUCTIVE TOXICOLOGY

As denoted by the subtitle, this portion of the research program is broader than HAAs, per se. The ACC is interested in better scientific understanding of developmental and reproductive toxicology, whether the mechanism involves hormones or other mediators/agents. If mechanisms were better understood, it would be possible develop more cost-effective methods and interpret existing experimental observations in terms of whether and to what extent these endpoints measure, correlate with, or predict adverse health effects. The latter is especially important in this revolutionary era of genomics.

The research to be discussed is managed by the ACC's LRI. In January 1999, the chemical industry initiated the LRI to sponsor research that is aligned with public health and environmental issues of highest priority to society, as well as the chemical industry. The ultimate goal of the LRI is to increase knowledge of the potential impacts that chemicals may have on the health of human and wildlife populations and the environment. Achieving this goal requires that the LRI provide a sound scientific foundation for input into the risk equation (hazard x exposure = risk).

Through the LRI, the members of ACC have committed to sponsor independent third-party research. Through guidance offered by scientists from industry, academia, and government, the research is providing valuable assistance to government in making risk assessment judgments about the health and environmental impacts of chemicals, and more certainty regarding those impacts for the public and manufacturers of those chemicals. This goal is far too broad for any one organization to tackle alone, so the LRI seeks programs that are complementary to or collaborative with those of others. The entire ACC LRI program is funded at a level of $25 million/year and contains a multiplicity of projects. The largest portion is on the endocrine and reproductive effects (about $7 million in 2001).

The LRI's research program is conducted independently at the CIIT Centers for Health Research (CIIT) in Research Triangle Park, NC, and at universities and other research institutes, typically through collaborations with other federal agencies and via competitive research contracts. LRI research contracts originate from requests for proposals (located on the LRI website and distributed to other websites as appropriate). Proposals receive independent, external peer review. Those proposals that are ranked excellent or very good in scientific merit are reviewed for relevance to the chemical industry and aims of the LRI. Funding decisions are then made. The LRI follows traditional research principles; the process is open. The principal investigators own the data, so they make decisions about the publication of their results. The LRI has no approval authority over publications. Investigators are strongly encouraged to present their results at scientific meetings and publish in the peer-reviewed literature. If for some reason the investigators decide not to publish, the LRI will make the investigators' final report available publicly, as written by the investigators.

The global LRI endocrine program was initiated with state-of-the-science documents (STOTS); there were about eight of them, one of which was on endocrine issues. International experts from academia, industry, and government were invited to come together and talk about the major research needs to understand the endocrine disruption hypothesis. The STOTS on the endocrine issue has been recently updated, and the update can be found on CEFIC's website. Many realized that there was more to reproductive and developmental toxicology than just endocrine issues. Therefore, a workshop of experts from all scientific sectors was convened in 2000 to identify research needs on reproductive and developmental toxicology. The results of this workshop also guided the program.

The goal of the ACC's LRI program on HAAs is to improve methods that can better characterize the potential reproductive and developmental effects of chemicals, not only in humans but also in wildlife, by mechanisms that are both dependent on and independent of the endocrine system. The key questions driving the program are: what are the underlying mechanisms involved in hormonally-mediated effects,

how should potential hazards of HAAs be evaluated, how should such data be used in risk assessment, to what degree does exposure to typical environmental levels of HAAs cause these effects, and how widespread is the actual or potential risk? The program discussed below is divided according to human or wildlife receptors.

Human Health Program

The LRI program emphasizes understanding mechanisms by which chemicals may interfere with reproduction and development. The LRI issued a joint Request for Applications with the U.S. National Institute of Environmental Health Sciences that called for exploratory research on the mechanism of action of developmental toxicants using advanced tools, such as genomics, model organisms, and transgenic animals. One of the objectives was to stimulate more basic biology experts to work on some toxicology endpoints to expand the science itself. In 2002, at least 12 grants were jointly funded.

Another large part of the program seeks to expand scientific understanding of how to extrapolate effects from animals to humans. Several of the projects utilize the phytoestrogen, genistein, as the model chemical. Genistein presents a unique opportunity because humans (and laboratory animals) are naturally exposed in their diets. Genistein is also similar to many synthetic HAAs in that it is a relatively weak estrogen. The LRI is sponsoring research to develop physiologically based pharmacokinetic models in pregnant and non-pregnant rats, including evaluation of lactational transfer. In addition, LRI- sponsored researchers are studying molecular mechanisms that might be involved (e.g., gene expression, receptor activities) in the effects of genistein. Researchers are also investigating the interactions of synthetic estrogens with genistein that is normally part of laboratory animal diets. Results may inform interpretations of the potential influence of background estrogens in diets on common laboratory animal tests of endocrine function.

The LRI program includes investigating mechanisms involved in the potential effects of chemicals on the development of the male reproductive system. This research involves a number of agents, including phthalates, bisphenol A, and several others. The research goal is to determine whether molecular effects or early markers (e.g., gene expression) can be linked to health outcomes in neonates and adults, (e.g., changes in developmental landmarks) so that the molecular events can be interpreted in terms of functional outcomes. Gaining more knowledge of the timing of exposure relative to effects is also a study objective.

Understanding mechanisms only has impact when data are integrated into scientifically advanced health risk assessment models. Towards this goal, the LRI is sponsoring the development of a biologically based dose-response model, based on some measurements as well as computational analyses, to elucidate the relationship between exposures to an estrogen compound and a heath outcome. The health outcome being analyzed is the defeminization of the female animal brain during the neonatal period. Another project is comparing pharmacokinetics of estrogen in rats and humans, again to inform the issue of extrapolation between animals and humans, linked by more knowledge of dosimetry.

A new Request for Proposal seeks research on maternal-fetal and maternal-offspring pharmacokinetics. A literature search revealed a dearth of information on pharmacokinetics of the late gestation and lactation periods. With increasing interest in animal models of development, knowledge of the pharmacokinetics involved is fundamental to using developmental toxicity data in human health risk assessments.

People and wildlife are often exposed to mixtures of chemicals, but chemical mixtures research is a rarity because it is so difficult to do. In his presentation at the Symposium, Dr. Zacharewski described his research with mixtures, some of which is supported by the LRI. In addition, the LRI is sponsoring research to develop new methods uniquely tailored toward the evaluation of environmentally relevant exposures to mixtures. Historically, chemical mixture studies have used techniques drawn from the drug interaction

literature, which involves exposure to a small number of chemicals at high, pharmacologically active dose levels. In contrast, exposure to environmental chemicals is characterized by larger numbers of chemicals, at low (often sub-threshold) concentrations. Identification of optimal study approaches, especially statistical study design, is an important component of the work.

Wildlife Program

One of the main goals of the LRI wildlife program is to better understand the relationship between findings in the laboratory and the field. When assessors have laboratory data only, they typically ask, "What would happen in the real world?" When field data are available, the typical question is "what is the biological plausibility and causative factors of the complex observation; can these effects be reproduced in the laboratory." Extrapolation from both "directions" is needed for improved assessments.

The LRI is partially sponsoring studies by EPA investigators who are developing and applying gene array technology. They are using frog models and fish models of natural populations to compare gene array changes to functional changes in these species.

Investigators in Canada are extending their endocrine-related research on whole ecosystems. They are dosing small experimental lakes with a synthetic estrogen, and evaluating changes in aquatic populations (fish, zooplankton, and some benthic invertebrates) relating organism- and population-level responses.

One set of investigators is developing a laboratory population of fence lizards as a potential model for assessing HAAs in reptiles. They are studying exposure of the adults as well as exposure of the eggs to identify the optimal model. Other investigators are developing field methods for measuring changes in bird mating and reproductive behaviors as related to hormones indicative of reproductive success and high body burdens of HAAs. The LRI is also supporting research to develop laboratory models with alligators, fish, and mussels that can be used to investigate mixtures.

SUMMARY

The ultimate goal of the ACC's LRI is to be an international partner in improving the science underlying risk assessment and management of HAAs and other reproductive toxicants. ACC's LRI research efforts are in line with, and responsive to, the reviews conducted by numerous governments and other authoritative scientific bodies in recent years, which pointed out the need for additional research in the area of HAAs. In responding to concerns that exposure to low levels of synthetic and naturally occurring chemicals in the environment may pose a risk to humans and wildlife, ACC's LRI is dedicated to fostering further research and scientific exchange of ideas as the foundation for sound and effective product stewardship and public policies.

The LRI's website, www.uslri.org, has far more details on each of the studies summarized above. The JCIA website, www.nikkakyo.org, and the CEFIC website, www.cefic.be, provide more information on their research programs on HAAs.

Q&A

Takei: Thank you, Dr. Graham. Is there any questions or comments from the audience?

Graham: I am 36 seconds early! You got 30 seconds.

Q: I have one question to you. I think we all know that ICCA is also very active in the high production volume work in the United States and elsewhere, and I was wondering if in your organization there is a link between these two activities in such a way that one might consider that a number of these HPV chemicals would be interesting chemicals also to look at from an endocrine disrupter viewpoint.

So any of the assays that we are now having available or are in the process of developing, does ICCA consider to include those in their screening of HPV chemicals?

Graham: Let me give a little background on the HPV, High Production Volume chemicals. There is a huge industry program in collaboration with governments around the world to test these compounds according to test methods that have been agreed to and according to a schedule. The question is will some of these new methods be incorporated into that testing.

The easy answer is I do not know. A lot depends on what the test methods are and what the negotiations would be. Some of this would be a year or two in advance. It is obviously important to understand the range of endpoints that may be affected by chemicals. I am sure those discussions would happen, but I am on the research side and so I really cannot comment further on that.

Q: To follow up a little bit on that, I know that part of the test battery that is considered for these HPV chemicals, which is usually referred to as the SIDS, Screening Information Data Set, does include nowadays a screening test for reproduction.

This is a test that is somewhat disputed in different parts of the world. It is appreciated very much here in Japan and to a large extent in the Unites States, but less appreciated in Europe. I thought maybe there has been some discussion within your organization of how to approach this particular element because reproduction is part of that HPV basic screening set to be considered anyway.

Graham: There might well be such things going on. I am not involved with the HPV program; I am involved with the research side of the house, so I do not know.

Takei: OK, thank you very much.

Graham: Well, thank you.

Adachi: My name is Adachi from the Ministry of the Environment. Thank you very much for being here with us today. I have two questions.

The ACC Project is characterized by open process. You stated that, if approved by the investigator, any results are made available to the public.

My first question is, could you be a little more specific about the way of opening the process?

The other thing I'd like to know is, if an investigator says "no," the results are not made available. Is it available to the public what sort of investigation was conducted?

I know I said two questions, but I have a third one. Is there any way to find out what sort of results are not made available as a result of the investigation?

Graham: Well, let me describe the process from the beginning to the end, briefly. We do work through the request for proposal; like if you look at our website, you will see requests for proposals along with instructions and what happens. We get proposals in from anybody, in fact, we fund internationally, it is not just for the United States, it is for any investigators that want to turn in proposals.

They get competitively reviewed by people who are independent, i.e., industry people do not serve as peer reviewers on the work. There is a confidentiality about the proposal. In other words, if one of the investigators here sends a proposal to us, we certainly give it to the reviewers, but we do not tell the whole world about your proposal.

Once we go thorough that and once we get a pile of proposals that are excellent or very good, then we do a relevance review and see how much money we have, how many can we fund, and we make the funding decision.

The funding is in terms of a research contract. What our contract specifies — and it is a legally binding contract — it says in there that the investigator owns the data, that the investigator does not need to give us copies before the work is published. We do say that when your work is accepted for publication, we want a copy; we want to know what you did.

We have no review rights; so the investigator can say whatever he wants and send if off to the journal. The journal's peer review system will certainly have some sort of comments, so it is independent.

You asked the question what happens if the investigator does not want to publish. We have in our contract a statement that we want you to publish in the peer review literature; we will pay the page charges, we will deal with that as part of the project.

But if the investigator says, No, I will not publish it, then we say that we have the right to take their final report and we will make their final report public, so we put the final report on our website. Now, we will not change it, we will not change the words in it, but we require a final report; the final payment is linked to the final report. So either the investigator publishes in the open literature or we will put it on our website; our preference is that it is going to be in the open literature.

Takei: Thank you very much.