Greetings RSESS Members:

This edition of the Regulatory and Safety Evaluation Specialty Section newsletter includes timely information from Carol Auletta on RSESS sponsored student awards, an update on Immunotoxicology guidelines from Pat Haley, and a thoughtful article from Bob Osterberg (our past President), Kimber White, and Robin Huff on the status of scientific and toxicology expertise at the FDA. My thanks to the authors for providing us with these articles… Happy reading!

“Change is in the Air —”

Many – perhaps most -- of us experience changes in our working and personal lives as an almost ‘continuous’ process. Things rarely seem to stay the same for very long, much as we might wish it was otherwise. Some of the recent changes in regulatory and safety evaluation have been challenging. For those of you with interest in the pharmaceutical industry there have been important recent changes in FDA leadership. You may have noted the recent announcements relating to the transfer of some of CBER’s responsibilities for review and evaluation of therapeutic protein products to CDER. Similar activities are occurring in other areas of the regulatory community, and I’m sure that our membership would be interested in hearing ‘insider’ perspectives and ideas (good and not-so) about these changes. If you would be interested in sharing your views in a brief article please contact any of your officers or just direct your ideas for an article to me at Harry_M_Olson@sandwich.pfizer.com or Denise Robinson-Gravatt at denise_robinson-gravatt@groton.pfizer.com.

This ‘constant change’ theme is true also for those of us serving as your RSESS officers and councilors this year. We are trying to anticipate some of the changes that have driven our discipline and the industries represented to assure that we meet your expectations for our Specialty Section for the coming year. During this summer we’ve had teleconference meetings to plan out some activities for this year, including one face-to-face meeting (scheduled for October 4) at SOT headquarters. The RSESS face-to-face meeting last year was great to keep us connected with SOT issues and helped us to focus our efforts on those topics likely to have broad interest. This October we will dedicate some time from our day jobs to focus on most (or all) of the following:

1. Student Awards for 2003
2. RSESS Budget (Rita Rose’s assistance here)
3. Newsletter development as a communication tool to membership
4. Roster of prospective candidates for ’03 RSESS officer vacancies
5. Annual meeting (’03 and ’04) program and reception planning
6. Vision and Mission for RSESS

We will be talking with you -- through the newsletter -- about these and other topics of interest throughout the remainder of 2002 and into 2003.

Best wishes,

Harry
**RSESS MISSION**

The mission of the Regulatory and Safety Evaluation Specialty Section (RSESS) of SOT is to promote the development of sound governmental policies and regulations based on contemporary scientific knowledge arising from the disciplines encompassed by toxicology. RSESS provides a forum for the interaction of SOT members to discuss the impact of regulations, guidelines, and guidance's on the practice of toxicology and the safety evaluation of food additives, nutraceuticals, therapeutic drug products and environmental, industrial and household chemicals, and other products of concern.

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**CONTRIBUTE TO THE LEADERSHIP PROCESS!! NOMINATIONS REQUESTED FOR RSESS OFFICERS**

The Regulatory and Safety Evaluation Specialty Section Executive Council requests your nominations for future officers for the section. Elections will be held before the end of 2002 for the following 2 positions:

Vice President Elect – position is one year in duration but implies a 4-year commitment with progression to Vice President, President and Past President.

Councillor – a 3-year position with progression to Secretary/Treasurer for the section during the third year.

Nominees must be members in good standing of both SOT and RSESS. The list of current members appears in the latest edition of the SOT Membership Directory for your reference. Please send any nominations to Harry Olson at Harry_M_Olson@sandwich.pfizer.com or to Denise Robinson at denise_robinson-gravatt@groton.pfizer.com as soon as possible!

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**Getting ready for the 2003 Annual Meeting! Student Travel Awards Available…..**

Are you, or do you know, a student who will be submitting a poster or making a presentation at the 2003 SOT meeting?

If so, you should know that the RSESS Specialty Section plans to continue our practice of providing travel awards to students with presentations applicable to regulatory toxicology or safety evaluation. In the past, awards of up to $500 have been presented to up to 4 students each year.

Applications consist of an application form, a copy of the abstract of work to be presented and a recommendation by the student’s academic advisor. Both undergraduate and graduate students are eligible. Forms and additional information will be available from the SOT web site later this year, after acceptances of abstracts. If you, or a student who is interested, need additional information, please contact:

Carol S. Auletta
Vice President, RSESS
AulettaC@princeton.huntingdon.com

We are looking forward to your applications !!!
Guidelines on Immunotoxicity Testing of New Chemical Entities, Consideration of a Conundrum

The required inclusion of immunotoxicology endpoints as part of routine preclinical toxicology programs in support of New Chemical Entities (NCEs) is now no longer a speculation. Final guidelines have been reviewed and issued by the Committee for Proprietary Medicinal Products (CPMP) and draft guidelines from the Food and Drug Administration (FDA) Taskforce on Immunotoxicity Testing and the Japanese Ministry of Health, Labor and Welfare (MHLW) are in final review with publication imminent for those of the FDA. How pharmaceutical companies are planning to respond to and/or incorporate the guidelines has stimulated much animated discussion and debate, including a Drug Information Association (DIA) workshop in Noordwijk, The Netherlands, November 2001.

Table 1. Immunotoxicology Endpoints Described in the Final Guidelines of the CPMP and the Draft Guidelines on Immunotoxicity Testing of the FDA

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CPMP</th>
<th>FDA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematology</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>Lymphoid organ weights</td>
<td>Spleen, thymus, local &amp; distant lymph nodes; bone marrow</td>
<td>√; not specified</td>
</tr>
<tr>
<td>Lymphoid organ histology</td>
<td>Spleen, thymus, local &amp; distant lymph nodes; bone marrow</td>
<td>Spleen, thymus, local &amp; distant lymph nodes; bone marrow</td>
</tr>
<tr>
<td>Bone Marrow Cellularity</td>
<td>√</td>
<td>Not specified</td>
</tr>
<tr>
<td>Immune cell phenotyping</td>
<td>√</td>
<td>Follow-up</td>
</tr>
<tr>
<td>NK cell activity</td>
<td>√</td>
<td>Follow-up</td>
</tr>
<tr>
<td>T-cell dependent antibody response</td>
<td>Follow-up; alternative to subset and NK analysis</td>
<td>Follow-up</td>
</tr>
<tr>
<td>Delayed type hypersensitivity</td>
<td>Follow-up</td>
<td>Follow-up</td>
</tr>
<tr>
<td>Lymphocyte proliferation</td>
<td>Follow-up</td>
<td>Follow-up</td>
</tr>
<tr>
<td>Macrophage function</td>
<td>Follow-up</td>
<td>Follow-up</td>
</tr>
<tr>
<td>Cytotoxic T-cell response</td>
<td>No</td>
<td>Follow-up</td>
</tr>
<tr>
<td>Cytokine production</td>
<td>No</td>
<td>Follow-up</td>
</tr>
<tr>
<td>Host resistance models</td>
<td>Follow-up</td>
<td>Follow-up</td>
</tr>
</tbody>
</table>

While the CPMP and FDA documents approach the discussion of immunotoxicity somewhat differently they share much in the resolution of the topic. The FDA document includes an extensive description of the immunologic basis for identification and selection of different assays, combined with very useful decision trees within the appendices. In comparison, the CPMP document is more concise and states in less equivocal terms specific aspects of study design, including study duration and endpoints to be evaluated. Table 1 lists the suggested endpoints from the two documents.

While the table shows many similarities, there are important differences that should be noted. From the table, it is apparent that the required tests are more explicitly described by the CPMP document. This difference might lead a sponsor to select the more conservative or detailed list (that of the CPMP) and operate on the assumption that it will also satisfy FDA expectations. This assumption, however, has not been tested.

However, rather than perusing these documents for a list of explicit requirements (remembering that these are guidelines, not requirements), it is better to look at the intents and overall goals of the documents. Both documents attempt to identify and state clearly the minimum expectations (routine assays) and then suggest scientific approaches to further delineate any signals of potential immunotoxicity risk in man. Both documents make unequivocal statements that all new drugs should be evaluated for immunosuppression. That said, both documents suggest that for most NCEs, a standardized approach incorporated in a routine toxicology study (i.e. routine 28-day toxicity study, as suggested by the CPMP), should suffice as the initial immunotoxicity screen. While the specifics of these ‘routine’ assays continue to provoke considerable debate, both documents agree that the interpretation of the initial immunotoxicity screen ‘should be based on an integrative analysis of changes…and other types of toxicity and the health status of the test animals’. In other words, identification of a risk for immunotoxicity should be based on sound, scientifically valid, integrative analyses of multiple toxicity endpoints. If such a risk is identified, then additional well-considered, scientifically valid studies should be selected on their likelihood to clarify, rather than confound, the issue.

Other points that reinforce building the immunotoxicological screen upon ‘a routine toxicology paradigm’ include incorporation of the dose-response relationship, the therapeutic margin (i.e. the relationship of the toxic dose to the pharmacologic dose), systemic exposure, reversibility of the immunotoxicity signals, and the identification of specific markers of the toxic response in multiple species.

Moreover, while both documents suggest preferred assays (there persists a debate on the identification of some assays as ‘preferred’), both documents agree that any assay of immune function for which there is a valid scientific rationale can be used. Indeed the ICH Expert Working Group on Harmonization of Regional Immunotoxicity Guidelines made the follow-
Guidelines on Immunotoxicity Testing (Cont.)

Thus, the wording of both documents, suggests that while there are preferred tests for evaluating immunocompetence, any scientifically valid approach should fulfill the needs of the regulatory agencies.

However, the documents also leave unanswered important questions that would be well served by international harmonization. For example, the criteria for selection of the dose(s) to be used for immunotoxicity testing not addressed in either document. This is of particular concern in the context of performing immunotoxicity assays within routine 28-day toxicity studies as suggested by the CPMP. In its recommendation, the CPMP does not specify if immunotoxicity testing need be done at all doses or, if only one dose group is selected for testing, how the selection of that dose should be made. Likewise, the FDA does not comment on dose selection for immunotoxicity testing. The ICH working group has made the recommendation that immunotoxicity testing be done below the maximal tolerated dose (MTD) to be sure that any changes observed are the result of primary effects on the immune system, rather than secondary to other toxicities. Clearly, carefully designed shorter term studies will have to be conducted to accurately identify the MTD prior to initiating the 28-day study, such as is the practice now.

The timing for inclusion of immunotoxicity testing is not discussed in the FDA document, while the CPMP guideline suggests that the studies be conducted during routine 28-day toxicity studies. In actuality, the CPMP requirements specify that the tests only be completed prior to marketing of the drug. Because of the high attrition rate of drugs during Phase I clinical trials, the DIA workshop recommended that functional immunotoxicity testing be carried out during Phase II development so as to minimize expenditure of unnecessary resources.

Another important question concerns how much additional resource will be required to include the suggested modifications to existing 28-day protocols. One can recognize vestiges of the original Tier I and Tier II immunotoxicity testing paradigms that rely on tried-and-true methods of organ weights, clinical pathology, gross pathology and histologic morphology as they apply to the immune system. What the CPMP guidelines suggest is taking these basic approaches a step further and requiring the weighing, gross examination and histologic examination of draining and distant lymph nodes as part of routine 28-day toxicity studies. While simple in concept, the accurate identification, trimming and weighing of non-routine lymph nodes (i.e., draining lymph nodes not currently collected in routine studies) is likely to generate highly variable data until standards are established and staff become adequately trained. Likewise, the accurate identification of the gross morphologic limits of the mesenteric lymph node in any species can be a significant challenge (ask any pathologist). Moreover, histologic examination of step-wise sections of lymph nodes is associated with significant morphologic variability depending on the orientation of the node within the block.

In addition to the more extensive evaluation of the lymphoid tissue morphology, the CPMP also recommends the inclusion of lymphocyte subset analysis, presumably by FACS analysis. Quantitative functional assays are also recommended including evaluation of T-dependent antigen-induced immune responses, and NK cell function, also presumably by FACS analysis. While initial calculations suggest adequate blood volumes can be collected from individual rats to accommodate all routine hematology, chemistry and coagulation assays, separate studies or groups of animals may have to be considered to collect adequate samples, especially for the T-dependent antigen-induced immune response. Moreover, because the majority of toxicologists are still reluctant to expose test animals to a neoantigen while attempting to evaluate general toxicity, the evaluation of the humoral immune response to a T-dependent antigen may require a separate study, or at least satellite groups of animals. In either case, if the assays are to be included in a standard 28-day toxicity study, then careful planning will be needed to collect the additional samples. In addition there is as yet no good agreement as to the most appropriate T-dependent antigen to be used in such studies. While the modified Jerne plaque assay for identification of anti-SRBC antibody-forming cells is one of the better known assays, it is also one of the most variable and difficult to standardize within a lab. KLH and tetanus toxoid are possible substitute antigens, but historical databases and experience are lacking.

Additional points of particular concern raised by the FDA, but not the CPMP documents, include: 1) evaluation of immune function in F1 generation animals if it is the intent to treat pregnant women, 2) evaluation of immune function if the drug is intended to treat patients for HIV or related immune disease (even if the initial screening does not indicate any alteration of immune function), and 3) evaluation of all drugs for hypersensitivity that are to be given by inhalation or topically. In the case of testing drugs destined for the treatment of HIV related immune disease, it is not clear how broadly this recommendation will apply. For example, will all drugs used to treat bacterial or fungal infections in HIV patients require such immune function testing?

One additional point identified in the FDA, but not in the CPMP document, is a guidance that if drug disposition studies
Guidelines on Immunotoxicity Testing (Cont.)

indicate that a drug is accumulating within the reticuloendothelial system, than functional analysis of macrophages should be done, even if there is no evidence of immunotoxicity emanating from the initial immunotoxicity screen. What is not clear is that if the drug is only accumulating in the Kupffer cells and not other macrophages, will the macrophage functional analysis mandate that Kupffer cells specifically be tested; if so this is not an exercise familiar to many.

In addition to the FDA and CPMP, the Japanese Ministry of Health, Labor and Welfare (MHLW) has also issued draft guidelines, that in contrast to the two guidelines already discussed, focuses on immunotoxicity testing per se, (i.e. immune suppression) and does not comment on augmented immune responses (i.e. hypersensitivity/allergy). Lymphocyte subset phenotyping or spleen immunohistochemistry in repeat-dose toxicity studies in rats are recommended as routine assays in the proposed MHLW guidelines. If immunotoxicity findings are noted in repeat-dose studies, a Tier I approach for immunotoxicity testing that includes a primary antibody response with or without a Natural Killer (NK) cell assay is employed. Immunotoxicity findings identified in the Tier I platform trigger additional tests described as Tier II. It should be noted that if immunotoxicity testing is selected as an ICH topic, the MHLW will not finalize their guidelines.

Perhaps the best road to successful fulfillment of the new guidelines would be the unbiased embracing of the regulatory agencies’ position that centers on the application of sound scientific enquiry coupled with special assays applied on a case-by-case basis to delineate and clarify the mechanisms of purported immunotoxicity. Coupled to this approach is the recognition that some assays, while fulfilling a minimal requirement may not actually contribute to an understanding of the problem (assays for assays sake is not good science). It is imperative that the respective laboratories/companies undertaking a given immunotoxicity study have a clear understanding of the limitations and biologic underpinnings of the assays they select (‘Choose, but choose wisely’ from ‘Indiana Jones the Last Crusade’).

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Director, Preclinical Sciences  
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Molecular and In Vivo Pharmacology: Striking the Right Balance

In the current scientific era, the explosion of genomic information is increasing the emphasis on research to identify genes and to then characterize the gene products and their cellular roles. Such emphasis has resulted in a reductionist approach to training in pharmacology research. The value of such research is clear. Voluminous quantities of new information are identifying potential new drug targets, providing mechanistic insights into drug action and disease mechanisms, explaining individual differences in response to xenobiotic exposures, providing strategies to identify critical interspecies differences, and improving models for assessing drug safety. Making further use of this information for drug discovery and development requires an understanding of physiology and in vivo experimentation. Yet, at a time when research institutions are taking an almost exclusively reductionist approach to pharmacology, industry laboratories and regulatory agencies are finding themselves in need of personnel capable of integrative in vivo experimentation and whole systems analyses. How this discordance will be resolved is beginning to be the subject of active inquiry.

Following discovery of a new gene and identification of its product, initial experiments are conducted in vitro to begin to understand the protein’s cellular function. If, based on these investigations, there is interest in the gene product as a therapeutic target, in vivo studies are conducted to explore the role the protein plays in the more complex whole animal. While the in vitro studies are useful hypothesis generating tools, the in vivo studies are necessary to probe the hypotheses so as to accurately predict the clinical consequences of pharmacological manipulation of the gene or protein. In the whole animal there is complex integration of multiple physiological systems, each of which has various feedback mechanisms. Such physiological complexity cannot be mimicked in vitro.

During drug development there are essentially two types of in vivo experiments, those designed to demonstrate the therapeutic utility of the drug and those designed to assure its safety. In vivo demonstration of efficacy is demanded by companies prior to embarking on costly clinical development. Numerous in vivo factors, such as compensatory physiological responses, bioavailability, tissue distribution patterns, and pharmacokinetics can impact a drug candidate’s viability. While drug candidates are designed to be selective for a particular target, absolute specificity is rarely if ever achieved. Therefore, in vivo experiments in relevant models are necessary to provide a comprehensive evaluation of potential toxicities.

Thus, modern drug development is a continuum from target identification, to in vitro testing, to in vivo pharmacological and toxicological evaluation. Yet because more and more academic institutions appear to be focusing almost exclusively on molecular and cellular research, the number of students receiv-
Molecular and In Vivo Pharmacology (Cont.)

The emphasis on physiology and related subjects is echoed in education initiatives underway at the Center for Drug Evaluation and Research (CDER) at FDA. It has been recognized for many years that fewer and fewer reviewer applicants possess extensive or even sufficient in vivo experience. CDER is investigating ways to supplement the training of younger review scientists through courses offered at local academic institutions or through agency sponsored courses with heavy emphasis on whole animal physiology. In addition, the possibility of short-term sabbaticals at government, academic or contract laboratories is being investigated as a means of providing “hands-on” experience. It is unclear what steps industry will take to address the diminishing pool of scientists with in vivo experience, although in the UK a unique paradigm exists whereby the British Pharmacological Society administers a fund to support in vivo training of pharmacology students, with funding provided by major pharmaceutical firms. In addition, the Welcome Trust has funded an initiative to promote integration of in vitro and in vivo research.

Thus, initiatives to ensure that pharmacologists and toxicologists receive sufficient in vivo training and are capable of making integrative data assessments are beginning to emerge. Success will require academia, government and industry to work together to ensure that a proper balance of molecular and in vivo training of pharmacologists is achieved. Only by striking this balance can the maximal benefit to the public health be derived from the drug development and review process.

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Chair, Education Subcommittee
Pharmacology and Toxicology Coordinating Committee
Center for Drug Evaluation and Research
Food and Drug Administration

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Acting Associate Director of Pharmacology
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NOTIFICATION OF CHANGE IN BY-LAWS FOR RSESS

Earlier this year, a change in the RSESS by-laws was recommended by the RSESS Executive Council to assist with succession planning for the section. Just as there is currently succession planning for the officers of Vice-President Elect, Vice-President, President and Past-President, it was recommended that the Secretary/Treasurer position be restructured as a succession to the position of Councilor. The Councilor would proceed to the position of Secretary/Treasurer after the experience gained during 3 years as a Councilor to the section, and thus would be more familiar with the working of the Section and its officers. This will allow for greater continuity of direction and planning in the section. This recommendation was voted on by the RSESS membership along with the officers’ ballot in December 2001 and accepted.

By-Law Change: Beginning in 2002, each year, the Councilor completing his or her term as Councilor will assume the position of Secretary/Treasurer for the Regulatory and Safety Evaluation Specialty Section for the next year.