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Division of American Home Products Corporation

WORLDWIDE REGULATORY AFFAIRS

August 8, 2001

Dockets Management Branch (HFA-305)
Food and Drug Administration
5630 Fishers Lane, Room 1061
Rockville, MD 20852

Re: Docket No. 01D-0177 - FDA Draft Guidance for Industry on Immunotoxicology Evaluation of Investigational New Drugs

Dear Sir/Madam:

Wyeth-Ayerst Research, a division of American Home Products Corporation, is submitting written comments on the draft guidance for industry entitled "Immunotoxicology Evaluation of Investigational New Drugs" (66 FR 24145, May 11, 2001).

Wyeth-Ayerst is a major research-oriented pharmaceutical company with leading products in the women's health care, cardiovascular, central nervous system, anti-inflammatory, infectious disease, hemophilia, and oncology categories, and is also a major manufacturer of preventative vaccines.

American Home Products is one of the world's largest research-based pharmaceutical and health care products companies. It is a leader in the discovery, development, manufacturing, and marketing of prescription drugs and over-the-counter medications. It is also a global leader in vaccines, biotechnology, and animal health care.

We are submitting the enclosed comments in duplicate. Wyeth-Ayerst appreciates the opportunity to comment on the above-mentioned draft guidance for industry.

Sincerely,

Roy J. Baranello, Jr.
Assistant Vice President
Worldwide Regulatory Affairs

Enclosure

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General Comments:

The draft guidance document dated April 2001 provides an overview of the agency's views and expectations in terms of (1) assessing nonclinical immunotoxicity and (2) responding to potential toxicities associated with the immune system. The draft guidance document advocates use of conventional tox/path endpoints (clinical hematology, chemistry, histopathology, etc.) in repeated-dose toxicity studies for initial evaluation of immunotoxic potential. If immune system-related findings are noted, the draft guidance document indicates that follow-up studies are needed using a case-by-case approach in order to better understand the potential safety implications of those findings.

The need and/or specifics of the follow-up studies should take into account the pharmacologic activity and intended clinical use of the compound being developed. The draft guidance document implies that additional follow-up immunotoxicity testing be focused on those compounds (1) that demonstrate evidence of unintended immunotoxicity, (2) that have known toxicological, historical or structural data indicative of potential immunotoxicity, or (3) whose recommended use (e.g., labeled for chronic use, use in pregnancy) poses potentially serious consequences if the compound is immunotoxic

The draft guidance document indicates that unintended immune system-related findings should be followed-up with additional immunotoxicity testing to better evaluate the potential significance in terms of safety assessment and to assist in defining potential risk/benefit ratios. It should be noted, however, that many of the suggested follow-up tests, while based on sound scientific grounds, have not been rigorously evaluated with regard to their ability to predict human toxicity. In addition, many of the follow-up evaluations will likely not be conducted in strict compliance with GLP regulations.

In general, the draft document provides a framework that the pharmaceutical industry is expected to work within to evaluate the concerns of the agency with regard to nonclinical assessment of immunotoxicity. Comments on specifics of the guideline are as follows:

Specific Comments:

Lines 53-56: The statement "... any decrease of more than 40 percent in total lymphocytes ... or 75 percent in granulocyte counts ... could be significant ..." should preferably be replaced with "... any decrease of more than 40 percent in total lymphocytes ... or 75 percent in granulocyte counts *in rodents* ... could be significant ..."

It is important to recognize that interpretation of leukogram findings is distinct between rodents and other species. Indeed, the whole issue of what constitutes a biologically significant change in much of the testing proposed in the draft document appears open to interpretation.

Lines 63-64: "Where possible, dose comparisons to clinical use should be based on relative body surface areas."

We recommend that this sentence be restated as, "When human exposure data is not yet available, depending on the nature of the compound, it may be appropriate to base clinical dose comparisons on relative body surface area, since this often better reflects actual systemic exposure when scaling from animals to humans."

Lines 64-65: In a discussion of various factors to consider when assessing immune system parameters, these lines state, "Other considerations include (1) ... other toxicities ..."

It seems warranted to further emphasize here that any additional systemic toxicity is critical to interpretation. Even alterations in food consumption, changes in body weight without other gross or histologic findings can cause significant changes in immune response patterns (Moriguchi et al., 1989). It would also be helpful to include a recommendation on dose selection for immunotoxicity testing to avoid such confounding additional toxicities. At least for follow-up testing, dosing at the high end of clinically relevant doses or at a dose not to exceed the NTEL might be most preferable.

Lines 66: In continued discussion of factors to consider with immunotoxicity testing, this line states, "... (3) reversibility of immunotoxic effects."

It is warranted to note that immune system alterations, especially functional impairment, may be readily reversible, but can take more than the standard 4 weeks for resolution (Talmadge et al., 1994). In particular, resolution of suppressed antigen-specific immune functions may require a 6 to 8-week recovery period in rodents.

Lines 78-89: "The pharmacological effects of the drug should be considered (e.g., where adverse immune changes result indirectly from effects of the drug on the central nervous system or the hypothalamic-pituitary-adrenal axis)."

A recommended restatement of this is " The pharmacological effects of the drug should be considered (e.g., where *alterations of the immune response* result indirectly from effects of the drug on the central nervous system or the hypothalamic ...)."

Drugs with unintended indirect pharmacologic effects on immune responses through neuroendocrine pathways include common compounds such as synthetic and natural estrogens, progestins, and thyroid hormones. Whether the immunomodulatory effects induced by these compounds are adverse is debatable, although further testing of their immune system effects may be indicated and/or beneficial.

Lines 86-90: The term "reticuloendothelial tissues" should be replaced with *"phagocytic cells"* or *"lymphoreticular tissues"*.

Also, information in this paragraph seems unclear. Most often a compound that is retained in tissues, and especially within phagocytic cells is either benign, or behaves as an adjuvant in stimulating immune cell responses. Yet, standard testing of macrophage function generally evaluates for suppression of non-specific phagocytosis and killing. There is little in the literature to support that localized retention of a pharmaceutical (non-metal or metalloid) compound in

lymphoreticular tissues alters these general innate immune responses in vitro. In addition, it is unclear whether enhancement, as well as suppression of phagocytic or killing activity would be considered "adverse".

Lines 96-100: "Indicators of immunosuppression can be observed in standard nonclinical toxicology studies and include:

- Evidence of myelosuppression such as pancytopenia, anemia, leukopenia, lymphopenia, throbocytopenia, or other blood dyscrasias ..."

We recommend the above be revised as follows, "Indicators of immunosuppression can be observed in standard nonclinical toxicology studies and *may* include:

- Evidence of myelosuppression, *decreased erythrocyte mass or peripheral blood leukocyte, platelet or lymphocyte counts or blood dyscrasias ..."*

The terms "anemia" and "leukopenia" are considered clinical terms based on historical reference ranges. These terms may not be truly appropriate in nonclinical studies since alterations in these parameters are determined relative to those of pretest values or a vehicle control group. In addition, immunotoxicity is an uncommon cause of alterations in erythrocytes or platelets, and if it is a consideration, it most often is indicative of immunostimulation through immune-mediated anti-rbc or platelet activity. Thus, changes in erythrocyte or platelet counts should probably be considered indicators of immunosuppression only when concomitant effects on other immune cell or tissues are similarly suggestive. Further, in nonclinical toxicology testing of pharmaceuticals, decreases in erythrocyte mass, leukocyte count, etc. most often do not represent a true blood "dyscrasia".

Line 107: We recommend, "Increased incidence of tumors" is changed to, "Increased incidence of certain tumor types (e.g., lymphoma/leukemia)".

Data obtained with known immunosuppressive agents does not demonstrate an increased incidence of most tumor types, suggesting that the relationship between tumorigenesis and immunosuppression is not generalizable.

Line 109: Consider replacing the words "immunoglobulin levels" to *"total serum globulin level"*, as only the latter is typically evaluated in conventional clinical pathology testing. The significance of decreased globulin levels is generally comparable to that of decreased immunoglobulin levels.

Lines 126-128: For clarification, additional text (in italics) is recommended for this sentence, "These sites are the gut-associated lymphoid tissues (GALT) for oral administration and the *regional* draining lymph nodes *(if they can be identified during macroscopic examination)* for intramuscular, intradermal or subcutaneous administration."

Often a specific single or localized set of lymph nodes draining an IM, ID, or SQ site is not readily identifiable and can vary in number and location between species. Therefore, the lymph

nodes of choice for each site need to be specifically defined for each species, or regional nodes that are macroscopically enlarged from individual animals, should be acceptable for this analysis.

Lines 166-168: "Consultation with FDA staff can be helpful during study design, but as a general practice, cell surface phenotype determinations should be made on splenocytes and, when practical, on circulating white blood cells."

A suggested change to this sentence is "... but as a general practice, cell surface phenotype determinations should be made on splenocytes *or peripheral blood lymphocytes*."

Phenotyping of peripheral blood lymphocytes, compared with that of splenocytes, is usually no less, and often a more practical procedure, and may be more sensitive and specific in detecting true immunotoxic effects (Woo et al., 1990). In addition, especially in rodents, splenic lymphocyte subset distribution and functions are distinct and should not be considered directly representative of those of other peripheral lymphoid tissues and blood. In particular, extramedullary hematopoiesis and, in rats, age-dependent density of "suppressors cells" unique to the spleen can significantly affect distribution and function of splenocytes (Flaherty et al., 1997). In addition, the draft guidance document indicates that phenotyping of all circulating white blood cells be determined. However, if a routine complete peripheral blood differential is performed, there should be no reason to pursue phenotyping of all peripheral blood leukocytes (other than lymphocytes) except under special circumstance.

Lines 173-174: The proportion of NK cells in spleen or in peripheral blood of rodents is very low, and there is little in the literature to suggest that determination of absolute numbers of NK cells in these tissues is apt to yield important immunotoxic effects. Therefore, to include these cells in phenotype analyses seem unwarranted.

Lines 183-184: "Both percentage and absolute cell counts can be determined by a single method ..."

It may be useful to add here that absolute cell counts for spleen are preferably based on gram weight rather than whole spleen.

Lines 202-204: The statement that ELISA measurement of anti-SRBC antibodies is "not a true measure of immune function" is mistaken. The immune system must function in order to generate antigen specific antibodies in serum, and it can be contended that the measurement of antigen specific serum antibodies is a more appropriate measure of the systemic response to a T-cell dependent antigen, since it measures the specific antibody where it is intended to exert its protective effect in terms of host resistance.

In addition, ELISA assays in general provide greater flexibility in characterizing the antigen specific antibody response (kinetics, isotypes, etc.). SRBCs are used as a T-cell dependent antigen only for historic reasons and the assays used were developed prior to the advent of ELISA methodologies. Indeed, sponsors should be encouraged to develop other T-dependent antigens (KLH, OVA, DNP-OVA, etc) to evaluate "immune function", which are likely to provide a more robust and consistent response and/or assay.

Lines 234-235: "True antigens are digestible by antigen-presenting cells (APC)", might be more clearly stated as, "*Complete* antigens are processed for presentation by antigen presenting cells (APC)."

Lines 257-259: It is unclear here under what circumstances large molecular weight compounds should be assessed for antigenicity.

Lines: 265-267: While it might be useful to develop an ELISA or blastogenic assay for the purpose of evaluating hypersensitivity responses to a compound-derived hapten, determination of the precise hapten-protein complex(es) which initiate the specific response can be extremely difficult. In addition, grading an in vitro reaction to derived antigenic moiety(ies) based on mixed lymphocyte proliferative response or antibody production generally tends to have very limited sensitivity and specificity, and be poorly predictive of an in vivo response. Thus, consideration of this testing methodology seems only rarely plausible and appropriate, and then only for very selective compounds.

Line 282: Could be more appropriately stated as such, "Small molecular weight drugs are *potentially antigenic* if they bind directly to proteins ..."

Lines 313-314: Only a few laboratories have investigated cytokine gene or protein expression patterns in local draining lymph nodes of mice exposed cutaneously to well-established potent respiratory sensitizers (e.g., TMA). Results of these studies have been mixed with expression analysis of only a single cytokine (IL-4) showing potential in distinguishing possible respiratory sensitizers (Dearman et al., 1999; Vandebriel et al., 2000). It is also well documented that the cytokine patterns elicited in an induced hypersensitivity response can significantly vary with mouse strain. Thus, to suggest evaluation of cytokine expression patterns as a means of testing for compounds of unknown potential as respiratory sensitizers seems premature.

Lines 354-363: Is it correct to interpret this paragraph as applying exclusively to halothane-related compounds without general application? If so, we recommend revising the wording to make this more clear.

Lines 371-374: In part because of animal welfare issues, the Buehler Assay and Guinea Pig Maximization Test (GPMT) are now questionably the most common methods currently used for evaluating dermal sensitizing potential of a compound. Because of both animal welfare issues and scientific rationale (e.g., non-specific immunostimulation associated with the use of Freund's Adjuvant and sodium lauryl sulfate, incidence(s) of false positives and usually a lack of a dose-response pattern) with these two assays, it seems prudent to more strongly encourage other approaches to test for sensitizing potential, such as the LLNA and modified LLNA assays.

Lines 491-494: It seems unclear at what age these parameters (histopathology and hematology) should be evaluated in the F_1 generation.

Hematologic values change significantly within the first 4 post-natal weeks of age in rodents, and broad variability in these parameter values among prepubertal offspring is common. Thus, the most preferable age to evaluate toxic effects on hematologic parameters in rodents may be

after puberty. However, histologic evaluation of the thymus might preferably be performed in the neonate or juvenile animal.

Lines 512-518: The choice of assay and/or antigen to assess immune function should not be restricted to the SRBC plaque assay. The use of other T-cell dependent antigens (KLH, OVA, DNP-OVA, or others) should be allowed, if not encouraged.

Lines 520-522: The Coombs assay has not been well studied nor validated as a test for immune-mediated hemolytic anemia (IHA) in rodents. Therefore, it may be warranted to further note that to test for toxin-induced IHA, the compound or metabolite may need to be included in vitro, as well as being administered in vivo to achieve adequate drug concentrations (e.g., as with penicillins). See also comment for lines 96-100 pertaining to anemia in non-clinical studies.

Lines 562-566: Scientific rationale argues against testing the potential of a compound to act as a Th-2-promoting respiratory sensitizer by evaluating the compound's capacity to induce a Th-1-like contact hypersensitivity reaction. Thus far, cutaneous application for induction in testing for respiratory sensitizers has been used to differentiate compounds that can induce both skin (Th-1-like) and respiratory (Th-2-like) hypersensitivity reactions from those that induce only contact hypersensitivity. Compounds with strictly respiratory sensitizing potential have not been adequately evaluated by the LLNA assay methodology. Thus, while testing inhalants for cutaneous hypersensitivity may be warranted, the method should not be considered valid for determining respiratory sensitizing potential. In addition, the unmodified murine LLNA does not discriminate between skin sensitizers and irritants. While, a variety of reported modifications for this latter purpose have been reported, these modifications have not yet been standardized nor validated.

The attached algorithms indicate follow-up studies to pursue depending on results of routine toxicity testing. The second flowchart (attachment) (see **Box IIA-C**) indicates that if immunosuppression is suggested in initial tests, follow-up evaluation should include one or more of the classic assays, such as immunophenotyping, the plaque assay, the NK assay, in vitro blastogenesis, host resistance models, or cutaneous hypersensitivity testing. However, these various assays are designed to determine very different aspects of an immunotoxic effect. For example, immunophenotyoing, the plaque assay, the NK cell assay, and cytotoxic T-cell functional assays are designed to determine whether, and which specific cells type(s) are affected. Whereas, host-resistance, and to some degree, cutaneous hypersensitivity assays, are of greatest benefit in predicting "clinical relevance" of a finding. If evaluating the mechanism of immunotoxicity is the goal, then host-resistance models and cutaneous hypersensitivity assays that non-specifically test a broad spectrum of immune cell types and their functions would seem inappropriate. However, determining the clinical relevance of an immunomodulatory effect is an important goal and should be considered if compound-related immune system effects are suggested by either tier I or tier II testing. This flowchart could be titled "FOLLOW-UP STUDIES TO CONSIDER FOR EXPLORING POSSIBLE MECHANISMS AND CLINICAL RELEVANCE OF IMMUNOTOXICITY".

The second flowchart (see **Box V**) also states that "There are no established assays that reliably assess potential for autoimmunity and acute systemic hypersensitivity. The [PLNA] has only a relatively small database ..." It is clear from this statement and lines 444-446 that the PLNA is

not considered a reliable nor validated assay for regulatory purposes, and therefore to include it in the flowchart as a recommended follow-up test appears inappropriate.

References:

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