

two animals in accordance with paragraph (f)(2) of this section. After application (2 to 5 minutes) the treated areas of the animals must be washed with a mild soap and water. The amounts of test substance recovered in the washes must be determined to assess the effectiveness of removal by washing.

(B) Unless precluded by corrosiveness, the test substance must be applied and kept on the skin for a minimum of 6 hours. At the time of removal of the covering, the treated area must be washed following the procedure as outlined in the dermal washing study. Both the covering and the washes must be analyzed for residual test substance. At the termination of the studies, each animal must be sacrificed and the treated skin removed. An appropriate section of treated skin must be analyzed to determine residual radioactivity.

(2) *Inhalation.* A single (or more if needed) concentration of test substance must be used in this portion of the study. The concentration should be selected in accordance with paragraph (f)(2) of this section. Inhalation treatments are to be conducted using a "nose-cone" or "head-only" apparatus to prevent absorption by alternate routes of exposure. If other inhalation exposure conditions are proposed for use in a chemical-specific test rule, justification for the modification must be documented. A single exposure over a defined period must be used for each group—a typical exposure is 4–6 hours.

[65 FR 78815, Dec. 15, 2000]

§ 799.9780 TSCA immunotoxicity.

(a) *Scope.* This section is intended to meet the testing requirements under section 4 of TSCA. This section is intended to provide information on suppression of the immune system which might occur as a result of repeated exposure to a test chemical. While some information on potential immunotoxic effects may be obtained from hematology, lymphoid organ weights and histopathology (usually done as part of routine toxicity testing), there are data which demonstrate that these endpoints alone are not sufficient to predict immunotoxicity (Luster *et al.*, 1992, 1993 see paragraphs (j)(8) and (j)(9)

of this section). Therefore, the tests described in this section are intended to be used along with data from routine toxicity testing, to provide more accurate information on risk to the immune system. The tests in this section do not represent a comprehensive assessment of immune function.

(b) *Source.* The source material used in developing this TSCA test guideline is the OPPTS harmonized test guideline 870.7800 (June 1996 Public Draft). This source is available at the address in paragraph (j) of this section.

(c) *Definitions.* The following definitions apply to this section.

Antibodies or immunoglobulins (Ig) are part of a large family of glycoprotein molecules. They are produced by B cells in response to antigens, and bind specifically to the eliciting antigen. The different classes of immunoglobulins involved in immunity are IgG, IgA, IgM, IgD, and IgE. Antibodies are found in extracellular fluids, such as serum, saliva, milk, and lymph. Most antibody responses are T cell-dependent, that is, functional T and B lymphocytes, as well as antigen-presenting cells (usually macrophages), are required for the production of antibodies.

Cluster of differentiation (CD) refers to molecules expressed on the cell surface. These molecules are useful as distinct CD molecules are found on different populations of cells of the immune system. Antibodies against these cell surface markers (e.g., CD4, CD8) are used to identify and quantitate different cell populations.

Immunotoxicity refers to the ability of a test substance to suppress immune responses that could enhance the risk of infectious or neoplastic disease, or to induce inappropriate stimulation of the immune system, thus contributing to allergic or autoimmune disease. This section only addresses potential immune suppression.

Natural Killer (NK) cells are large granular lymphocytes which non-specifically lyse cells bearing tumor or viral antigens. NK cells are up-regulated soon after infection by certain microorganisms, and are thought to represent the first line of defense against viruses and tumors.

T and *B* cells are lymphocytes which are activated in response to specific antigens (foreign substances, usually proteins). B cells produce antigen-specific antibodies (see the definition for "antibodies or immunoglobulins"), and subpopulations of T cells are frequently needed to provide help for the antibody response. Other types of T cell participate in the direct destruction of cells expressing specific foreign (tumor or infectious agent) antigens on the cell surface.

(d) *Principles of the test methods.* (1) In order to obtain data on the functional responsiveness of major components of the immune system to a T cell dependent antigen, sheep red blood cells (SRBC), rats and/or mice¹ shall be exposed to the test and control substances for at least 28 days.² The animals shall be immunized by intravenous or intraperitoneal injection of SRBCs approximately 4 days (depending on the strain of animal) prior to the end of the exposure. At the end of the exposure period, either the plaque forming cell (PFC) assay or an enzyme linked immunosorbent assay (ELISA) shall be performed to determine the effects of the test substance on the splenic anti-SRBC (IgM) response or serum anti-SRBC IgM levels, respectively.

(2) In the event the test substance produces significant suppression of the anti-SRBC response, expression of phenotypic markers for major lymphocyte populations (total T and total B), and T cell subpopulations (T helpers (CD⁴) and T cytotoxic/suppressors (CD⁸)), as assessed by flow cytometry, may be performed to determine the effects of the test substance on either splenic or peripheral-blood lymphocyte populations and T cell subpopulations. When this study is performed, the appropriate monoclonal antibodies for the species being tested should be used. If the test substance has no significant

effect on the anti-SRBC assay, a functional test for NK cells may be performed to test for a chemical's effect on non-specific immunity.³ For tests performed using cells or sera from blood (ELISA or flow cytometry), it is not necessary to destroy the animals, since immunization with SRBCs at 28 days is not expected to markedly affect the results of other assays included in subchronic or longer-term studies (these tests are discussed in the reference under paragraph (j)(7) of this section). The necessity to perform either a quantitative analysis of the effects of a chemical on the numbers of cells in major lymphocyte populations and T Cell subpopulations by flow cytometry, or a splenic NK cell activity assay to assess the effects of the test compound on non-specific immunity shall be determined on a case-by-case basis, depending upon the outcome of the anti-SRBC assay.

(e) *Limit test.* If a test at one dose level of at least 1,000 mg/kg body weight (or 2 mg/L for inhalation route of exposure) using the procedures described for this study produces no observable toxic effects or if toxic effects would not be expected based upon data of structurally related compounds, then a full study using three dose levels might not be necessary. Expected human exposure may indicate the need for a higher dose level.

(f) *Test procedures—(1) Animal selection—(i) Species and strain.* These tests are intended for use in rats and/or mice. Commonly used laboratory strains shall be employed.⁴ All test animals shall be free of pathogens, internal and external parasites. Females shall be nulliparous and nonpregnant. The species, strain, and source of the animals shall be identified.

¹ If absorption/distribution/metabolism/excretion (ADME) data are similar between species, then either rats or mice may be used for the test compound in question. If such data are lacking, both species should be used.

² Because there is a fairly rapid turnover of many of the cells in the immune system, 28 days is considered sufficient for the purposes of the anti-SRBC tests.

³ When these optional tests are included, the phenotypic or NK cell analyses may be performed at 28 days of exposure, or at a later timepoint if ADME data suggest that a longer exposure is more appropriate.

⁴ The study director shall be aware of strain differences in response to SRBC. For example, if the B₆C₃F₁ hybrid mouse is used in the PFC assay, a response of 800-1,000 PFC/10⁶ spleen cells in control mice should be the minimally acceptable PFC response.

(ii) *Age/weight.* (A) Young, healthy animals shall be employed. At the commencement of the study, the weight variation of the animals used shall not exceed $\pm 20\%$ of the mean weight for each sex.

(B) Dosing shall begin when the test animals are between 6 and 8 weeks old.

(iii) *Sex.* Either sex may be used in the study; if one sex is known or believed to be more sensitive to the test compound, then that sex shall be used.

(iv) *Numbers.* (A) At least eight animals shall be included in each dose and control group. The number of animals tested shall yield sufficient statistical power to detect a 20% change based upon the interanimal variation which may be encountered in these assays.

(B) To avoid bias, the use of adequate randomization procedures for the proper allocation of animals to test and control groups is required.

(C) Each animal shall be assigned a unique identification number. Dead animals, their preserved organs and tissues, and microscopic slides shall be identified by reference to the animal's unique number.

(v) *Husbandry.* (A) Animals may be group-caged by sex, but the number of animals per cage shall not interfere with clear observation of each animal. The biological properties of the test substance or toxic effects (e.g., morbidity, excitability) may indicate a need for individual caging.

(B) The temperature of the experimental animal rooms shall be at 22 ± 3 °C.

(C) The relative humidity of the experimental animal rooms shall be between 30 and 70%.

(D) Where lighting is artificial, the sequence shall be 12 hrs light, 12 hrs dark.

(E) Control and test animals shall be maintained on the same type of bedding and receive feed from the same lot. The feed shall be analyzed to assure adequacy of nutritional requirements of the species tested and for impurities that might influence the outcome of the test. Rodents shall be fed and watered *ad libitum* with food replaced at least weekly.

(F) The study shall not be initiated until the animals have been allowed an adequate period of acclimatization or

quarantine to environmental conditions. The period of acclimatization shall be at least 1 week in duration.

(2) *Control and test substances.* (i) The test substance shall be dissolved or suspended in a suitable vehicle. Ideally, if a vehicle or diluent is needed, it shall not elicit toxic effects or substantially alter the chemical or toxicological properties of the test substance. It is recommended that an aqueous solution should be used. If solubility is a problem a solution in oil may be used. Other vehicles may be considered, but only as a last resort.

(ii) One lot of the test substance shall be used, if possible, throughout the duration of the study, and the research sample shall be stored under conditions that maintain its purity and stability. Prior to the initiation of the study, there shall be a characterization of the test substance, including the purity of the test compound and if technically feasible, the name and quantities of any known contaminants and impurities.

(iii) If the test or positive control substance is to be incorporated into feed or another vehicle, the period during which the test substance is stable in such a mixture shall be determined prior to the initiation of the study. Its homogeneity and concentration shall also be determined prior to the initiation of the study and periodically during the study. Statistically randomized samples of the mixture shall be analyzed to ensure that proper mixing, formulation, and storage procedures are being followed, and that the appropriate concentration of the test or control substance is contained in the mixture.

(3) *Control groups.* (i) A concurrent, vehicle-treated control group is required.

(ii) A separate untreated control group is required if the toxicity of the vehicle is unknown.

(iii) A positive control group with a known immunosuppressant (e.g., cyclophosphamide) shall be included in the study. A group of at least eight animals shall be given the immunosuppressive chemical.

(4) *Dose levels.* (i) In repeated-dose toxicity tests, it is desirable to have a

dose-response relationship and a no observed immunotoxic effect level. Therefore, at least three dose levels and a negative control shall be used, unless a limit test is performed as specified under paragraph (e) of this section.

(ii) The highest dose level shall not produce significant stress, malnutrition, or fatalities, but ideally should produce some measurable sign of general toxicity (e.g., a 10% loss of body weight).

(iii) The lowest dose level ideally shall not produce any evidence of immunotoxicity.

(5) *Administration of the test substance.*

(i) The test substance, vehicle, or positive control substance shall be administered for at least 28 days for the anti-SRBC assay. The route of administration of the test material will usually be oral; however, this shall be determined by the likely route of occupational or indoor exposure. Therefore, under certain conditions, the dermal or inhalation route of exposure may be more relevant for the study. All animals shall be dosed by the same method during the entire experimental period.

(ii) If the test substance is administered by gavage, the animals are dosed with the test substance ideally on a 7-days-per-week basis. However, based primarily on practical considerations, dosing by gavage on a 5-days-per-week basis shall be acceptable. If the test substance is administered in the drinking water, or mixed directly into the diet, then exposure shall be on a 7-days-per-week basis.

(A) For substances of low toxicity, it is important to ensure that when administered in the diet, the quantities of the test substance involved do not interfere with normal nutrition. When the test substance is administered in the diet, either a constant dietary concentration in parts per million (ppm) or a constant dose level in terms of the animal's body weight shall be used; the alternative used should be specified.

(B) For a substance administered by gavage, the dose shall be given at approximately the same time each day, and adjusted at intervals (weekly for mice, twice per week for rats) to maintain a constant dose level in terms of the animal's body weight.

(iii) If the test substance is administered dermally, use paragraphs (f)(5)(iii)(A) through (f)(5)(iii)(D) of this section.

(A) *Dose levels and dose selection.* (1) In this test, it is desirable to determine a dose-response relationship as well as a NOEL. Therefore, at least three dose levels plus a control and, where appropriate, a vehicle control (corresponding to the concentration of vehicle at the highest dose level) group should be used. Doses should be spaced appropriately to produce test groups with a range of toxic effects. The data should be sufficient to produce a dose-response curve.

(2) The highest dose level should elicit signs of toxicity but not produce severe skin irritation or an incidence of fatality which would prevent a meaningful evaluation. If application of the test substance produces severe skin irritation, the concentration may be reduced, although this may result in a reduction in, or absence of, other toxic effects at the high dose level. If the skin has been badly damaged early in the study, it may be necessary to terminate the study and undertake a new one at lower concentrations.

(3) The intermediate dose levels should be spaced to produce a gradation of toxic effects.

(4) The lowest dose level should not produce any evidence of toxic effects.

(B) *Preparation of animal skin.* Shortly before testing, fur should be clipped from not less than 10% of the body surface area for application of the test substance. In order to dose approximately 10% of the body surface, the area starting at the scapulae (shoulders) to the wing of the ileum (hipbone) and half-way down the flank on each side of the animal should be shaved. Shaving should be carried out approximately 24 hrs before dosing. Repeated clipping or shaving is usually needed at approximately weekly intervals. When clipping or shaving the fur, care should be taken to avoid abrading the skin which could alter its permeability.

(C) *Preparation of test substance.* (1) Liquid test substances are generally used undiluted, except as indicated in paragraph (f)(5)(iii)(A)(2) of this section.

(2) Solids should be pulverized when possible. The substance should be moistened sufficiently with water or, when necessary, a suitable vehicle to ensure good contact with the skin. When a vehicle is used, the influence of the vehicle on toxicity of, and penetration of the skin by, the test substance should be taken into account.

(3) The volume of application should be kept constant, e.g. less than 300 μ L for the rat; different concentrations of test solution should be prepared for different dose levels.

(D) *Administration of test substance.* (1) The duration of exposure should be at least for 90 days.

(2) The animals should be treated with test substance for at least 6 hrs/day on a 7-day per week basis. However, based on practical considerations, application on a 5-day per week basis is acceptable. Dosing should be conducted at approximately the same time each day.

(3) The test substance should be applied uniformly over the treatment site.

(4) The surface area covered may be less for highly toxic substances. As much of the area should be covered with as thin and uniform a film as possible.

(5) During the exposure period, the test substance should be held in contact with the skin with a porous gauze dressing. The test site should be further covered with nonirritating tape to retain the gauze dressing and the test substance and to ensure that the animals cannot ingest the test substance. Restraints may be used to prevent the ingestion of the test substance, but complete immobilization is not recommended.

(iv) If the test substance is administered by the inhalation route, use the procedures under paragraphs (e)(2), (e)(3), (e)(6), (e)(8), (e)(9), and (e)(10) of 40 CFR 799.9346. The exposure time for the anti-SRBC test shall be at least 28 days.

(6) *Observation period.* Duration of the observation period shall be at least 28 days.

(7) *Observation of animals.* (i) Observations shall be made at least once each day for morbidity and mortality. Appropriate actions shall be taken to

minimize loss of animals to the study (e.g., necropsy of those animals found dead and isolation or euthanasia of weak or moribund animals).

(ii) A careful clinical examination shall be made at least once a week. Observations shall be detailed and carefully recorded, preferably using explicitly defined scales. Observations shall include, but not be limited to: evaluation of skin and fur, eyes and mucous membranes; respiratory and circulatory effects; autonomic effects, such as salivation; central nervous system effects, including tremors and convulsions, changes in the level of motor activity, gait and posture, reactivity to handling or sensory stimuli, grip strength, and stereotypes or bizarre behavior (e.g., self-mutilation, walking backwards).

(iii) Signs of toxicity shall be recorded as they are observed, including the time of onset, degree and duration.

(iv) Food and water consumption shall be determined weekly.

(v) Animals shall be weighed immediately prior to dosing, weekly (twice per week for rats) thereafter, and just prior to euthanasia.

(vi) Any moribund animals shall be removed and euthanized when first noticed. Necropsies shall be conducted on all moribund animals, and on all animals that die during the study.

(vii) The spleen and thymus shall be weighed in all animals at the end of the study.

(g) *Immunotoxicity tests*—(1) *Functional tests.* Either a splenic PFC assay or an ELISA shall be used to determine the response to antigen administration.

(i) *Antibody plaque-forming cell (PFC) assay.* If the antibody PFC assay is performed, the criteria listed under paragraphs (g)(1)(i)(A) through (g)(1)(i)(F) of this section shall be adhered to. Assays described in the references under paragraphs (j)(2) and (j)(4) of this section may be used.

(A) The T cell-dependent antigen, SRBC, shall be injected intravenously or intraperitoneally, usually at 24 days

after the first dosing with the test substance.⁵ Although the optimum response time is usually 4 days after immunization, some strains of test animal may deviate from this time point. The strain to be used shall be evaluated for the optimum day for PFC formation after immunization.

(B) The activity of each new batch of complement shall be determined. For any given study, the SRBCs shall be from a single sheep, or pool of sheep, for which the shelf life and dose for optimum response has been determined.

(C) Modifications of the PFC assay described in paragraph (g)(1)(i) of this section exist and may prove useful; however, the complete citation shall be made for the method used, any modifications to the method shall be reported, and the source and, where appropriate, the activity or purity of important reagents shall be given. Justification or rationale shall be provided for each protocol modification. Discussions of modifications of the PFC assay are available in the references under paragraphs (j)(5), (j)(6), and (j)(10) of this section.

(D) Samples shall be randomized and shall be coded for PFC analysis, so that the analyst is unaware of the treatment group of each sample examined.

(E) Spleen cell viability shall be determined.

(F) The numbers of IgM PFC per spleen, and the number of IgM PFC per 10⁶ spleen cells shall be reported.

(ii) *Immunoglobulin quantification.* As an alternative to a PFC assay, the effects of the test substance on the antibody response to antigen may be determined by an Enzyme-Linked Immunosorbent Assay (ELISA). Comparison between the PFC and ELISA assays for immunotoxicity assessment are discussed in the references under paragraphs (j)(5), (j)(6), and (j)(10) of this section. Test animals shall be immunized with SRBCs as for the PFC assay. IgM titers in the serum of each test animal shall be determined (usually 4 days after immunization). As

⁵ If the SRBCs are administered by the intraperitoneal route, the study director should be aware that a low percentage of animals may not respond because the antigen was accidentally injected into the intestinal tract.

with the PFC assay, the optimum dose of SRBCs and optimum time for collection of the sera shall be determined for the species and strain of animal to be tested. Several methods are described in the reference under paragraph (j)(11) of this section).

(iii) *Natural killer (NK) cell activity.* The methods described in the reference under paragraph (j)(3) of this section may be used to demonstrate the effects of at least 28 days of exposure to a test substance on spontaneous cytotoxic activity. In this assay, splenocytes from treated and untreated test animals are incubated with ⁵¹Cr-labeled YAC-1 lymphoma cells. The amount of radiolabel released from the target cells after incubation with the effector cells for four hrs is used as a measure of NK cytotoxicity. The following points shall be adhered to when using the NK cell assay:

(A) Assay controls shall be included to account for spontaneous release of radiolabel from target cells in the absence of effector cells, and also for the determination of total release of radiolabel.

(B) Target cells other than YAC-1 lymphoma cells may be appropriate for use in the assay. In all cases, target cell viability shall be determined.

(C) Modifications of the protocol exist that may prove useful. However, complete citation shall be made to the method used. Modifications shall be reported, and where appropriate, the source, activity, and/or purity of the reagents should be given. Justification or rationale shall be provided for each protocol modification.

(2) *Enumeration of splenic or peripheral blood total B cells, total T cells, and T cell subpopulations.* The phenotypic analysis of total B cell, total T cell, and T cell subpopulations from the spleen or peripheral blood by flow cytometry should be performed after at least 28 days of dosing; this may be performed at a later timepoint, if ADME data suggest that a longer exposure is more appropriate. If an exposure period longer than 28 days is used, then these tests may be performed in conjunction with subchronic (ninety day oral, dermal, or inhalation) toxicity studies, when

these studies are required. Methods described in the references under paragraphs (j)(1) and (j)(5) of this section may be used.

(h) *Data and reporting*—(1) *Treatment of results*—(i) Data shall be summarized in tabular form, showing for each test group the number of animals at the start of the test, the number of animals showing effects, the types of effects and the percentage of animals displaying each type of effect.

(ii) All observed results, quantitative and incidental, shall be evaluated by an appropriate statistical method. Any generally accepted statistical methods may be used; the statistical methods including significance criteria shall be selected during the design of the study.

(2) *Evaluation of study results*. The findings of an immunotoxicity study shall be evaluated in conjunction with the findings of preceding studies and considered in terms of other toxic effects. The evaluation shall include the relationship between the dose of the test substance and the presence or absence, and the incidence and severity of abnormalities, including behavioral and clinical abnormalities, gross lesions, identified target organs, body weight changes, effects on mortality and any other general or specific toxic effects. A properly conducted test shall provide a satisfactory estimation of a no-observed-effect level. It may indicate the need for an additional study and provide information on the selection of dose levels.

(3) *Test report*. In addition to the reporting requirements as specified under 40 CFR part 792, subpart J, the following specific information shall be reported. Both individual and summary data should be presented.

(i) The test substance characterization shall include:

- (A) Chemical identification.
- (B) Lot or batch number.
- (C) Physical properties.
- (D) Purity/impurities.
- (E) Identification and composition of any vehicle used.

(ii) The test system shall contain data on:

- (A) Species, strain, and rationale for selection of animal species, if other than that recommended.
- (B) Age, body weight data, and sex.

(C) Test environment including cage conditions, ambient temperature, humidity, and light/dark periods.

(D) When inhalation is the route of exposure, a description of the exposure equipment and data shall be included as follows:

(1) Description of test conditions; the following exposure conditions shall be reported:

(i) Description of exposure apparatus including design, type, volume, source of air, system for generating aerosols, method of conditioning air, treatment of exhaust air and the method of housing the animals in a test chamber.

(ii) The equipment for measuring temperature, humidity, and particulate aerosol concentrations and size should be described.

(2) Exposure data shall be tabulated and presented with mean values and a measure of variability (e.g., standard deviation) and include:

(i) Airflow rates through the inhalation equipment.

(ii) Temperature and humidity of air.

(iii) Actual (analytical or gravimetric) concentration in the breathing zone.

(iv) Nominal concentration (total amount of test substance fed into the inhalation equipment divided by volume of air).

(v) Particle size distribution, calculated mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD).

(vi) Explanation as to why the desired chamber concentration and/or particle size could not be achieved (if applicable) and the efforts taken to comply with this aspect of the section.

(E) Identification of animal diet.

(iii) The test procedure shall include the following data:

(A) Method of randomization used.

(B) Full description of experimental design and procedure.

(C) Dose regimen including levels, methods, and volume.

(iv) Test results should include the following data:

(A) Group animal toxic response data shall be tabulated by species, strain, sex, and exposure level for:

(1) Number of animals exposed.

(2) Number of animals showing signs of toxicity.

(3) Number of animals dying.
 (B) Individual animal data shall be presented, as well as summary (group mean data).

(C) Date of death during the study or whether animals survived to termination.

(D) Date of observation of each abnormal sign and its subsequent course.

(E) Absolute and relative spleen and thymus weight data.

(F) Feed and water consumption data, when collected.

(G) Results of immunotoxicity tests.

(H) Necropsy findings of animals that were found moribund and euthanized or died during the study.

(I) Statistical treatment of results, where appropriate.

(i) *Quality control.* A system shall be developed and maintained to assure and document adequate performance of laboratory staff and equipment. The study shall be conducted in compliance with the 40 CFR Part 792—Good Laboratory Practice.

(j) *References.* For additional background information on this test guideline, the following references should be consulted. These references are available for inspection at the TSCA Non-confidential Information Center, Rm. NE-B607, Environmental Protection Agency, 401 M St., SW., Washington, DC, 12 noon to 4 p.m., Monday through Friday, except legal holidays.

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(6) Ladics, G.S., Smith, C., Heaps, K., and Loveless, S.E. Evaluation of the humoral immune response of CD rats following a 2-week exposure to the pesticide carbaryl by the oral, dermal, or inhalation routes. *Journal of Toxicology Environmental Health*. 42:143-156 (1994).

(7) Ladics, G.S., Smith, C., Heaps, K., Elliot, G.S., Slone, T.W., and Loveless, S.E. Possible incorporation of an immunotoxicological functional assay for assessing humoral immunity for hazard identification purposes in rats on standard toxicology study. *Toxicology*. 96:225-238 (1995).

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(10) Temple, L., Kawabata, T. T., Munson, A. E., and White, Jr., K. L. Comparison of ELISA and plaque-forming cell assays for measuring the humoral immune response to SRBC in rats and mice treated with benzo[a]pyrene or cyclophosphamide. *Fundamental Applied Toxicology*. 21:412-419 (1993).

(11) Temple, L., Butterworth, L., Kawabata, T.T., Munson, A.E., and White, Jr., K.L. Eds. Burleson, G.R., Dean, J.H., and Munson, A.E. ELISA to Measure SRBC Specific Serum IgM: Method and Data Evaluation. Vol. 1. *Methods in Immunotoxicology* (Wiley-Liss, Inc., New York, 1995) pp. 137-157.