Frequently Asked Questions for OncoLogic™

1. What are the scientific bases of the rules used to develop OncoLogic™? OncoLogic™ uses mechanism-based structure-activity relationships (SAR) analysis, based on rule packages developed jointly by the Office of Pollution Prevention and Toxics of the U.S. Environmental Protection Agency (EPA) and an expert system developer, LogiChem, Inc. The rule packages were incorporated into decision trees that are used along with user input for SAR analysis. Cancer data from the following sources were used to develop the rule packages: (a) six-volume series of monographs entitled 'Chemical Induction of Cancer' [1-5]; (b) International Agency for Research on Cancer (IARC) monograph series; (c) U.S. National Cancer Institute (NCI)/National Toxicology Program (NTP) technical report series; (d) U.S. Public Health Service publication series 149 entitled 'Survey of Compounds Which Have Been Tested for Carcinogenic Activity'; and (e) non-classified chemical industry and U.S. EPA research data. Publicly available scientific literatures and external domain experts were also used/consulted whenever necessary.

2. What methods are used by OncoLogic™ to evaluate potential carcinogenicity? OncoLogic™ uses two different methods to predict potential carcinogenicity, structural (SAR) analysis, and functional analysis [6, 7, 8]. Structural analysis makes use of mechanism-based SAR analysis, which involves comparison with structurally related compounds with known carcinogenic activity, identification of structural moieties or fragments that may contribute to carcinogenic activity through a perceived or postulated mechanism, and evaluation of the modifying role of the remainder of the molecule to which the structural moiety/fragment is attached. The structural analysis arm consists of four modules, including the Organics module, Metals module, Polymers module, and Fibers module. Functional analysis integrates available mechanistic/non-cancer studies on the chemical in order to predict the potential for the chemical to be a tumor initiator, promoter, or progressor. Results from the functional analysis can be used to provide support to the results of the structural analysis, or can be used as an independent method of analysis. The structural and functional analyses must be performed separately.

3. How has OncoLogic™ been peer reviewed and validated? The details of the validation of OncoLogic™ have been discussed [6, 7]. Essentially, beyond internal validation and crosschecking, external peer reviewing and prospective validation were conducted. OncoLogic™ was peer reviewed at the developmental stage by external domain experts and, after completion of versions 2.0 and 4.0, by two international peer review panels of domain experts. In addition, the scientific bases of rule packages for a number of classes of chemicals were published in peer-reviewed open literature (e.g., 9, 10, 11). The OncoLogic™ team participated in two international, prospective predictive exercises sponsored by NTP/NIEHS to evaluate the capabilities of various methods to predict the outcome of cancer bioassays several years before the studies were completed. In the first exercise, focusing on 1 of 8

aromatic amines, OncoLogic™ achieved a high degree of accuracy [6]. In the second exercise on 30 chemicals of diverse structure, the OncoLogic™ approach [12] was rated as one of the best performers [13]. A recent external validation by U.S. FDA [14] showed that, within the limitations of the method, the predictive accuracy of OncoLogic™ exceeded 90% for the batch of chemicals the Agency was interested in. It should be cautioned, however, that the predictive accuracy of OncoLogic™ is expected to vary from batch to batch depending upon the structural diversity of chemicals relative to the underlying knowledge basis of OncoLogic™.

4. What are the major strengths and limitations of OncoLogic™? The major strengths of OncoLogic™ include the following: (a) OncoLogic™ does well in predicting potential carcinogenicity for chemicals that can be entered and evaluated in the system and that are, therefore, within the domain of applicability of the system; (b) OncoLogic™ predictions are backed by a mechanistic understanding/rationale and are capable of generating testable hypotheses; (c) OncoLogic™ uses a wealth of knowledge, incorporating decades of cancer research and the practical experience of a team of domain experts, in predicting the carcinogenic potential of chemicals by SAR analysis; and (d) OncoLogic™ is a flexible system capable of incorporating both chemical and biological information to predict potential carcinogenicity of a variety of chemicals. Along with these strengths, OncoLogic™ also has a few weaknesses that users should be aware of, including (a) OncoLogic™ is primarily designed to predict potential carcinogenicity of industrial chemicals and therefore may not work as well for pharmaceuticals; (b) there is no batch-mode function for entering several chemicals into OncoLogic™ at once; (c) chemical structures or SMILES structures cannot be imported from another file format into OncoLogic™; and (d) in order to use OncoLogic™ correctly, the user must have a basic knowledge of organic chemistry and ability to place chemicals in the appropriate chemical class.

5. I am not familiar with methods used for predicting carcinogenicity. Can I still use OncoLogic™? Yes, the main requirement is that you are familiar with the basic concepts of organic chemistry since the user must be able to place the chemical into the correct chemical class.

6. What information will I need to input a chemical into OncoLogic™? Necessary input information depends on the type of chemical. Inputs may include chemical name, CAS number, or structure, and chemical, biological, and mechanistic information (e.g., physicochemical properties, chemical stability, route of exposure, bioactivation and detoxification, genotoxicity, and other supportive data) critical to the evaluation of carcinogenic potential. OncoLogic™ prompts the user when any of this information is required for the specific chemical in question.

7. What type of output will I receive from OncoLogic™? Output information will include a prediction of the carcinogenic potential of the chemical, expressed semi-quantitatively (i.e., low, marginal, low-moderate, moderate, moderate-high, or high), and the underlying scientific rationale. The six concern levels are defined as follows: Low: Unlikely to be carcinogenic; Marginal: Equivocal or marginal carcinogen, or carcinogenic only with doses at or exceeding the maximum tolerated dose, or by a mechanism not relevant in humans; Low-Moderate: Likely to be weakly carcinogenic toward a single species/target, or carcinogenic at relatively high doses; Moderate: Likely to be a moderately active carcinogen toward one or more species/targets; Moderate-High: Highly likely to be an active carcinogen toward one or more species/target, or a

potent carcinogen at moderate or relatively high doses; and High: Highly likely to be a potent carcinogen, even at relatively low doses, or carcinogenic toward multiple species/targets.

8. Can I view sample chemicals for the chemical classes listed in OncoLogic™? Yes, sample chemicals are provided for many of the chemical classes in OncoLogic™. To view sample chemicals, highlight a chemical class, and then press "F1" on your keyboard. Refer to the OncoLogic™ User's Manual for a description of using OncoLogic™ for different types of chemicals, including a description of the required data input fields. Tutorials for sample fibers, polymers, metals, and organics compounds are available in OncoLogic™ User's Manual and in the Quick Start Tutorial.

9. If there is not an appropriate chemical class in OncoLogic™ for the chemical, can I still evaluate the potential carcinogenicity of the chemical using OncoLogic™? If an appropriate chemical class is not available, potential carcinogenicity cannot be evaluated using SAR analysis by OncoLogic™. For most cases, absence of an appropriate class/structure in OncoLogic™ provides suggestive, but not definitive, evidence of low cancer concern. If mechanistic/non-cancer studies are available, potential carcinogenicity can be evaluated using the functional analysis arm of OncoLogic™, instead of the structural analysis arm.

10. My chemical can be entered into more than one chemical class. How should I evaluate potential carcinogenicity? Review the OncoLogic™ User's Manual for tips on each chemical class, and then run the chemical through OncoLogic™ to get a concern for the chemical for each chemical class. Depending upon your needs, it may be appropriate to select one or the other concern (e.g., use the highest concern level as the overall concern for potential carcinogenicity of the chemical), or use the range.

11. I found the chemical class, but cannot draw the exact structure of my chemical. If the chemical in question cannot be drawn exactly, you must ensure that the chemical that you can draw contains all of the functional groups present in the chemical in question, or that can be included given the capabilities of OncoLogic™. In some cases, it may be necessary to use your best judgment to determine whether the chemical in question may have a similar, greater, or reduced potential for carcinogenicity compared to the chemical that can be drawn in OncoLogic™.

12. How do I add substituents or atoms when drawing a chemical structure? After selecting the type of substituent or atom to add, place the cursor just ahead of where you want the substituent to appear. Once a yellow box appears, click your mouse to add the substituent at the position of the box. Use "Escape" on your keyboard to select other substituents or atoms to add to the structure.

13. What is meant by the term reactive functional group (RFG), as it is used in OncoLogic™? How do I determine whether or not I should enter a functional group as an RFG? In OncoLogic™, RFG refers to a group of atoms, together forming a functional group (e.g., acrylamide, acrylate), which is reasonably anticipated to undergo chemical reaction. Refer to the OncoLogic™ User's Manual for additional information on RFGs. To determine whether or not to enter a functional group as an RFG, first, determine whether the functional group is

already listed in OncoLogic™ in the 'RFG Selection' screen under Oxygen, Nitrogen, Sulfur, Halogen, Other Heteroatom, or No Heteroatom, depending upon the atoms in the functional group. If not, and the functional group is potentially reactive, select "Unlisted Groups", and then enter the name of the functional group, the level of concern for the functional group, and its stability.

14. On the 'Water Solubility' screen in the polymers module, I entered "5" since the weight percent solubility of the polymer is 5%, but received the error message "invalid entry". What am I doing wrong? The water solubility should be entered as a decimal instead of as a percentage. The weight percent solubility should be entered as "0.05", not "5".

15. When using the polymers module, how do I calculate the reactive functional group equivalent weight (FGEW)? FGEW is the number average molecular weight divided by the number of functional groups of that type on the polymer. The following formula can be used to calculate FGEW: FGEW = Formula weight of the functional group x 100 Weight percent of the functional group in the polymer

16. How do I display help information for the various screens in OncoLogic™? There are "Help" buttons available within most of the screens in OncoLogic™, or you can hit the "F1" key to automatically display the help menu.

17. Why was the Code Number that I entered not accepted? The Code Number must be unique, and must consist of only letters and numbers. Names with other characters, spaces, or dashes will not be accepted by the program.

18. Do I need to save the Code Number and Substance Identification number that I entered? Yes, keep track of the Code Number for each chemical that is evaluated in OncoLogic™. The Code Number is the record identifier that you will need to view input data, print and view reports, and save/delete records. The Substance Identification number is for your own records and is not used by OncoLogic™.

19. Can I view input data for a chemical that I evaluated previously in OncoLogic™? Yes, from the main menu, select "Reports" and then "Display Report". Select the substance Code Number and then, at the 'Type of Report' screen, select "Data".

20. Can I view or print a justification report once I have closed the report or closed OncoLogic™? Yes. For viewing, select "Reports" at the main menu, and then "Justification" at the 'Type of Report' screen. For printing, select "Reports" at the main menu, and then "Print Report".

21. Where can I find additional information on the development and use of OncoLogic™? Refer to the information contained in the references at the end of this FAQ document for more details on OncoLogic™.

References

1. Arcos JC, Argus MF. Chemical Induction of Cancer: Structural Bases and Biological Mechanisms, Vol. IIA, Polycyclic Aromatic Hydrocarbons,New York: Academic Press, 1974.

2. Arcos JC, Argus MF. Chemical Induction of Cancer: Structural Bases and Biological Mechanisms, Vol. IIB, Aromatic Amines and Azodyes,New York: Academic Press, 1974.

3. Arcos JC, Woo YT, Argus MF. (in collaboration with Lai D.) Chemical Induction of Cancer: Structural Bases and Biological Mechanisms, Vol. IIIA, Aliphatic Carcinogens,New York: Academic Press, 1982.

4. Woo YT, Lai D, Argus MF, Arcos JC.Chemical Induction of Cancer: Structural Bases and Biological Mechanisms, Vol. IIIB, Aliphatic and Polyhalogenated Carcinogens, New York: Academic Press, 1985.

5. Woo YT, Lai D, Argus MF, Arcos JC. Chemical Induction of Cancer: Structural Bases and Biological Mechanisms, Vol. IIIC, Natural, Metal, Fiber, and Macromolecular Carcinogens, New York: Academic Press, 1988.

6.Woo YT, Lai DY, Argus MF, Arcos JC. Development of Structure-Activity Relationship Rules for Predicting Carcinogenic Potential of Chemicals, Toxicol. Lett. 1995; 79:219-228.

7. Woo YT, Lai DY. OncoLogic: A Mechanism-Based Expert System for Predicting the Carcinogenic Potential of Chemicals, In: Helma C, ed. Predictive Toxicology, Dekker, New York, 2005; C10.

8. Woo YT, Lai D, Argus MF, Arcos JC. An integrative approach of combining mechanistically complementary short-term predictive tests as a basis for assessing the carcinogenic potential of chemicals, Environ. Carcino. Ecotoxicol. Revs. 1998; C16:101-122.

9. Lai DY, Woo YT, Argus MF Arcos JC. Carcinogenic potential of organic peroxides: prediction based on structure-activity relationships (SAR) and mechanism-based short-term tests, Environ. Carcino. & Ecotoxicol. Revs.1996; C14:63-80.

10. Lai D, Woo YT, Argus MF, Arcos JC. Cancer risk reduction through mechanism-based molecular design of chemicals, In: DeVito SC, Garrett RL, eds. Designing Safer Chemicals, ACS Symposium Series 640, American Chemical Society, Washington, DC, 1996:62-73.

11. Woo YT., Lai DY. Aromatic amino and nitroamino compounds and their halogenated derivatives, In: Bingham E, Cohrssen B, Powell CH, eds. Patty's Toxicology,New York: Willey, 2001: 969-1106.

12. Woo YT, Lai D, Arcos JC, Argus M, Cimino M, DeVito S, Keifer L. Mechanism-based structure-activity relationship (SAR) analysis of carcinogenic potential of 30 NTP test chemicals, Environ. Carcino. Ecotoxicol. Revs. 1997; C13:139-160.

13. Benigni R, Zito R. The Second National Toxicology Program Comparative Exercise on the Prediction of Rodent Carcinogenicity: Definitive Results. Mutat. Res. 2004; 566:49-63.

14. Mayer J, Cheeseman MA, Twaroski ML. Structure-activity relationship analysis tools: validation and applicability in predicting carcinogens. Regul. Toxicol. Pharmaocl. 2008; 50:50-58.