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Project Summary

Final Report on the Evaluation of Four Toxic Chemicals in an *In Vivo/In Vitro* Toxicological Screen: Acrylamide, Chlordecone, Cyclophosphamide, and Diethylstilbestrol

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This document is an internal EPA report of research conducted across divisions within HERL and augmented by on-site contract support personnel. An *in vivo/in vitro* Toxicological Screen (Tox Screen) has been developed for screening large numbers of wastes for *biological activity*. Emphasis is placed on identifying a wide range of potential toxic responses by employing diverse test methods with toxic endpoints in mutagenesis/carcinogenesis, general toxicology, neurotoxicology, reproductive toxicology, teratology, and immunotoxicology. Oral administration of waste material is given to rats for 10 consecutive days after which the whole animal, body tissues and fluids are evaluated for toxicity. The Tox Screen is being validated to ensure that the protocol will be capable of detecting biological activity and to identify those *assays which most readily detect toxicity*. The most accurate and sensitive assays would be used as a Prescreen for the entire protocol. Results of the validation study with 4 toxic chemicals are included. Acrylamide produced its greatest effects in the neurotox assays and in mutagenesis. The reproductive assays were most sensitive in detecting the toxicity of chlordecone. The toxicity of cyclophosphamide was most readily identified by the immunological and mutagenesis tests. Diethylstilbestrol produced dose-response effects in all disciplines. The Tox Screen is currently being re-evaluated to answer questions concerning the length of exposure and the need to add and/or eliminate certain tests to increase sensitivity and accuracy. In addition, several new areas of research are being pursued in additivity/synergism/antagonism, chronicity and *in vitro* to *in vivo* extrapolation.

This Project Summary was developed by EPA's Health Effects Research Laboratory, Research Triangle Park, NC, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Technical Summary

An *in vivo/in vitro* Toxicological Screen (Tox Screen) employs methods designed to be rapid, inexpensive, capable of screening large numbers of wastes for biological activity, and identifying a wide range of potential toxic responses associated with each waste. The toxic endpoints to be identified include mutagenesis/carcinogenesis, general toxicology, neurotoxicology, reproductive toxicology, teratology, and immunotoxicology. The protocol of Tox Screen involves oral administration of waste material to rats for 10 consecutive days. At the end of the

10-day period, the whole animal and its body tissues and fluids are evaluated for toxicity. A major goal of Tox Screen is maximization of the potential health effects information obtained by exposing metabolically competent intact animals to complex waste mixtures.

Tox Screen is being validated through use of a series of compounds of known and defined toxicity. The purpose of the validation study is to ensure that the protocol will be capable of detecting biological activity associated with each compound and identifying those assays which most readily detect the toxic potential of the compounds. A selected number of the most accurate and sensitive assays might constitute a prescreening test for the entire protocol. The prescreening could then be used (with an abbreviated form of the *in vivo* exposure regime) as a rapid means of prioritizing waste samples to be evaluated in the full Tox Screen. It is important to note that a large number of compounds of known toxicity and no toxicity must be evaluated in the Tox Screen before bioassay tests for the prescreening would be initiated. These evaluation tests would constitute a validation study to establish the sensitivity, specificity, and accuracy of Tox Screen.

Initial trials of the validation study with acrylamide, chlordecone (kepone), cyclophosphamide, and diethylstilbestrol (DES) demonstrated the feasibility of the Tox Screen for detecting biological activity of chemicals.

Acrylamide, evaluated at five test concentrations ranging from 3.75 to 60 mg/kg in rats, produced its greatest toxic effects in motor activity, responses to acoustic stimuli (neurotoxicology), and in the induction of sister chromatid exchanges (mutagenesis). Most other tests resulted in positive responses at the highest concentration tested, a concentration which produced 30% lethality.

The reproductive assays were most sensitive in detecting the toxic potential of chlordecone (evaluated at 0.625 to 10.0 mg/kg in rats). Testicular sperm was markedly reduced, demonstrating that chlordecone is a reproductive toxin. This observation agrees with previously reported studies of sterility in chemical workers employed in chlordecone manufacturing in Hopewell, Virginia. Had this sample been an unknown hazardous waste mixture, the observed results would suggest the presence of a potential reproductive toxin and would indicate a need for further research.

The toxicity of cyclophosphamide (1.5 to 24 mg/kg in rats) was most readily identified by the immunological and mutagenesis tests. Most other tests responded at the highest concentration tested, resulting in 70% lethalities. Cyclophosphamide produced mutagenic metabolites in the urine of treated rats. These metabolites were detectable using the Salmonella histidine reversion assay. Increases in sister chromatid exchanges were identified in the bone marrow at each exposure concentration (1.5 to 24.0 mg/kg in rats). Dose-response relationships were established for each of the immunotoxicity tests with cyclophosphamide. Also, there were significant reductions in thymus and spleen weights at all concentrations tested. Body weight reductions were observed only at the two highest doses. These results indicate that generalized body weight loss is not predictive of potential immunotoxicity.

The exposure of animals to DES also resulted in the generation of a number of dose-response relationships in several areas. Almost all parameters evaluated in general toxicology, neurotoxicology, immunotoxicology, reproductive toxicology, and teratology were affected at the lowest concentration tested, 31.25 mg/kg. The only test category which did not detect the toxicity of DES was mutagenesis, which is not surprising since this toxin is not a bacterial mutagen nor does it induce SCEs.

The results of the validation study to date suggest that Tox Screen is capable of detecting a range of biological activity of the pure compounds as well as the type of activity associated with the pure compounds. Four compounds, however, are insufficient for establishing the required sensitivity (0.9), specificity (0.75), and accuracy specified by OSW or to select the best tests for the abbreviated prescreen. The number of compounds required to identify prescreen assays and to establish the accuracy of Tox Screen (with a 90% sensitivity) requires a minimum of 15 to 20 biologically active compounds for each of the six disciplines of Tox Screen (assuming no overlap in toxicity) and 15 to 20 control compounds without biological activity (assuming 100% overlap in toxicity across endpoints). This results in a total of 105 to 140 compounds. The evaluation of this many compounds may be a prohibitive task because of both time and cost limitations.

As a result, Tox Screen is being re-evaluated with respect to the treatment protocol and the toxicological assays which it employs. Several questions must be answered as part of this re-evaluation process and before validation of Tox Screen can be designed.

1. Is a 10-day exposure of a waste material adequate for predicting chronic effects? Should a 30- or 90-day exposure regime be employed?
2. Are there toxicity tests which could be added or deleted to improve the overall performance and sensitivity of the Tox Screen?
3. Can certain *in vivo* tests be replaced with *in vitro* tests to reduce cost without reducing sensitivity or accuracy?
4. Is 90% sensitivity and 75% accuracy actually required?

In addition to addressing the above questions, several new areas of research are being pursued as part of an ongoing developmental program in the evaluation of complex mixtures.

1. The development of short term *in vitro* genetic mutation and liver toxicity bioassays for the identification of hazardous waste samples possessing acute and chronic biological effects.
2. The development of new and improved *in vivo/in vitro* screening methods in the areas of liver toxicology, neurotoxicology, immunotoxicology, and developmental biology for addition to the integrated Tox Screen.
3. The investigation and resolution of some of the basic toxicological problems related to complex mixtures and the screening assay (i.e., acute vs. chronic effects and comparison of *in vivo* and *in vitro* methodologies).

This report covers a period from April 8, 1983 to June 22, 1985 and work was completed as of April 21, 1986.

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The complete report, entitled "Final Report on the Evaluation of Four Toxic Chemicals in an In Vivo/In Vitro Toxicological Screen: Acrylamide, Chlordecone, Cyclophosphamide, and Diethylstilbestrol," (Order No. PB 86-195 260/AS; Cost: $9.95, subject to change) will be available only from:

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