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Project Summary

Selection of Adduct-Forming Chemicals for Human Monitoring Studies

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The U. S. EPA, through its Environmental Monitoring Systems Laboratory-Las Vegas (EMSL-LV) and its Health Effects Research Laboratory-Research Triangle Park (HERL-RTP) has been exploring the feasibility of using biological markers to monitor exposure to environmental chemicals. Among the candidate biomarkers of exposure and effect are the adducts formed by reaction of carcinogenic electrophiles with DNA and/or protein.

In 1987, the staffs of HERL-RTP, EMSL-LV and Oak Ridge National Laboratory (ORNL) conducted a study designed to identify those chemical exposures of interest to the Agency which may, potentially, be effectively monitored using adduct-based techniques. The participants began by compiling a list of chemicals of known or suspected health hazards and for which the master list was then systematically evaluated for (1) the potential for adduct-formation *in vivo*, (2) the availability of supportive adduct research data, (3) the identifiability of population(s), and (4) the level of genetic activity. After considering all the relevant data, the participants selected and prioritized for further study a small group of chemicals considered to have the greatest potential for use in pilot, adduct-based, biological monitoring studies in human populations.

Introduction

The U. S. Environmental Protection Agency (EPA) has been exploring the feasibility of using biological markers to monitor exposure to environmental chemicals. Among the candidate biomarkers of exposure and effect are the adducts formed by reaction or carcinogenic electrophiles with DNA and/or protein. Certain carcinogens form electrophilic species that bind covalently to macromolecules such as DNA, RNA, and protein. New analytical methodologies including monoclonal antibody techniques, 32P postlabeling, and gas chromatography-mass spectrometry (GC/MS) have been developed to identify these chemical adducts. Application of these techniques may permit detection and quantitation of human exposure.

It was the objective of this project to (a) identify and rank adduct-forming chemicals of interest to the U.S. EPA, and (b) to select the most promising of those chemicals for further evaluation as candidates for use in future molecular epidemiological and exposure studies.

In 1987, the staffs of the Health Effects Research Laboratory-Research Triangle Park (HERL-RTP), the Environmental Monitoring Systems Laboratory-Las Vegas (EMSL-LV), and Oak Ridge National Laboratory (ORNL) met to discuss and conduct a study designed to identify those chemical exposures of interest to the Agency which may, potentially, be effectively monitored using adduct-based techniques.

Chemical Selection–The Initial Process

Section 2 describes the process employed to identify the chemicals that would be of interest to the Agency. The process

of chemical selection was divided into four steps based on the following four selection criteria: (1) interest to the Agency, (2) availability of supportive adduct research data, (3) availability of identified exposed population(s), and (4) genetic activity (i.e., genotoxicity). The first step was to compile a list of prioritized chemicals of interest to the EPA, i.e., chemicals which represent known or suspected health hazards and for which the potential for human exposure exists. The procedure used was to request a list of chemicals considered to be of high priority in terms of their potential health hazard from each of the EPA Program Offices.

The lists from each Program Office were prioritized, based on the number of times each chemical appeared. All of the lists were then combined into a common prioritized list. The final prioritized list (Appendix B) consisted of approximately 1,585 chemicals. The prioritized list of chemicals was then cross referenced with a list of 131 chemicals (Appendix C) that were known to form adducts based upon a review of the literature. Sixty-three of the chemicals listed in Appendix B had at least some data indicating that they form adducts. The potential candidates for further research and evaluation were primarily selected from this list of 63 compounds (Appendix D).

The second step was to identify supplemental sources of additional chemicals not listed in Appendix D. Appendix F lists 167 chemicals which, though not known to form adducts, are classified as known or suspected human carcinogens. Some of these chemicals may either (1) not yet have been tested for adduct-formation, or (2) the adducts they form were not yet detectable with the methods applied. Appendix G lists adduct-forming, suspect carcinogens that do not appear on the prioritized list. Most of these are drugs or research chemicals which are not subject to regulation by the EPA.

Initially, the lists of chemicals in Appendix D were screened for chemicals that could be eliminated a priori for one or more of the following reasons: (1) adduct formation by the chemical was considered unlikely for structural reasons, (2) the literature contained insufficient adduct information to support the chemicals's candidacy, or (3) the adduct formed by the chemical was a small alkylation product that lacked sufficient specificity for exposure monitoring. It should be kept in mind, that these alkylating agents may still be useful in screening studies to identify exposure to certain classes of chemicals (rather than to individual chemicals). In addition, some of these chemicals might display a specific adduct-binding pattern that would increase their specificity and sensitivity, thus making them useful for monitoring exposure.

Chemicals in Appendix D were marked with a plus sign (+) if (1) they were fairly well represented in the literature on adduct research, or (2) sufficient preliminary data (i.e., ongoing research) existed to support their candidate potential. In general, it was decided that the chemical(s) selected for the initial study should be one(s) that form chemical-specific adducts that can be identified with relative ease.

Section 2.2.3 describes the process used to identify and characterize potentially exposed human populations, the third step in the chemical selection process. The chemical list used in this step was a subset of a combined list of chemicals from two databases: (1) the GENETOX database and (2) the Genetic Activity Profile database. The selected chemicals tended to be those that were either known or considered likely to form adducts, based on chemical structure and available genotoxicity data. Appendix I lists the 253 chemicals that were subsequently examined and characterized for the availability of exposed populations for study.

The following criteria were used in scoring the chemicals on their human population exposure potential:

(1) population availability
(2) population size (best estimate)
(3) level of exposure
(4) quality of exposure history
(5) degree of confounding multiple exposures

Each criterion was assigned a score from 0 to 3 and the resulting overall score for a chemical was the product of the criterion scores. Only 33 chemicals had a score greater than zero and the remaining 195 chemicals received no score due to the absence of sufficient data.

The selected adduct-forming chemicals of interest to the Agency (Appendix D) were then re-examined in light of the relevant human population exposure data.

Section 2.2.4 describes the process of using genetic activity profiles in ranking the chemicals selected, the fourth step in the chemical selection process. The genetic toxicological activity of a compound, as measured by various bioassays, is relevant to both (1) the potential hazard it poses to human health and, hence, its interest to the EPA, and (2) the likelihood that the chemical will form detectable macromolecular adducts. Genetic activity profiles were particularly useful in rating those chemicals for which little adduct information was available.

Prioritization of Chemicals Selected for Further Study

Section 2.3 describes those chemicals that were not eliminated during the four steps of the chemical selection process described previously. Twenty- three chemicals listed in Table 1 were assigned high, medium, or low priority based on adduct data, current research status, population availability, and genetic activity. This section contains a discussion of the assigned priority of each chemical. An unclassified category for reconsideration was established for those chemicals for which the potential for study existed, but for which the information available at the time was insufficient to support a judgement.

Chemicals that were classified as mixtures were considered separately. While there is considerable interest in the potential for monitoring exposure to some of these chemicals, it was also recognized that (1) it would be difficult, due to the presence of confounding exposures, to attribute health effects to any specific component of a mixture (e.g., N-nitrosonornicatine in tobacco smoke), and (2) that potentially high background levels of adducts to ubiquitous chemicals such as benzene, benzo(a)pyrene, and 4-aminobiphenyl might preclude detection of all but the highest exposures.

Conclusions and Recommendations

The selection of Adduct-Forming Chemicals for Human Monitoring Studies document presents a strategy for selecting chemicals for further study. The selected chemicals in Table 1 should be further reviewed with particular emphasis on the following 13 chemicals: styrene, ethylene oxide, 4-4'methylene bis(2-chloroaniline) (MOCA), benzidine, vinyl formaldehyde, chlordane,nitropyrene,1,3-dichloropropene, and ethylene dichloride. It was considered that certain low priority chemicals (e.g., chloroform, 2,4,-toluene diisocyanate, benzyl chloride, o-toluidine, and malathion) might be of potential use in exposure monitoring studies based on measurement of protein adducts, but more research data were needed. It was recommended that adduct research on pesticides be reviewed. Finally, it was suggested that the chemical-adduct research data for each chemical be entered into a computer informa-

tional system. The efforts of the 1987 workgroup represented an initial step in the development of the Biomarkers Program of the U.S. EPA. Additional program development studies have, in the intervening time, followed up on the workgroup's initial conclusions and recommendations. The reports on these projects, which represent subsequent updates and refinements of the chemical selection process, are briefly described in Section 3.

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Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Table 1. *Prioritized Chemicals Selected for Further Study*

Number [a]	Chemical	Rating [b]	Data Summary
1. (11)	Styrene	H	Genetic activity = high. Good population data. Supportive adduct research studies.
2. (4)	Ethylene oxide	H	Genetic activity = high. Available populations: hospital sterilization workers and patients receiving treatment through the sterilized equipment used for cases such as renal dialysis, which provides more consistent dosing than bypass surgery. Numerous human studies using Hb adducts have been conducted.
3. (29)	4,4-Methylene bis (2-chloro-aniline) (MOCA)	M/H	Genetic activity = low. Available population. Active research support.
4. (18)	Benzidine	M/H	Genetic activity = high. Available population. Supportive adduct research data.
5. (2)	Vinyl chloride	M/H	Genetic activity = high. Population available, but numerous confounding exposures include vinyl bromide, acrylonitrile, 1,2-dichloroethane, 1,2 dibromoethane, ethylene halohydrins, and urethane. Also, exposure might be low and it must be determined if adducts would be formed at these levels. Additional research data is necessary for support.
6. (19)	Epichlorohydrin	M/H	Genetic activity = medium. Reasonable population in production workers. Needs further validation from animal studies.
7. (25)	Propylene oxide	M/H	Genetic activity = similar to ethylene oxide profile, although not as much information has been collected. Ubiquity could be a problem. Needs more supportive research data.
8. (3)	Formaldehyde	L/M	Genetic activity = high. Ubiquitous. Changed from H to L/M because it was believed that the separation of endogenous/exogenous adduct formation would be difficult and some reactivity may be reversible. (CIIT)
9. (15)	Acrylonitrile	L/M	Genetic activity = positive in vitro, questionable in humans. Available population.
10. (N/A)	Pentachlorophenol	L	Genetic activity = minimal. Availability of unconfounded exposed population is questionable. Indirect adduct formation. Additional data needed.
11. (N/A)	2,4-toluene diisocyanate	U/Ex	Genetic activity = inadequate information. Population available. Need more information, particularly on protein adducts as exposure biomarkers.
12. (6)	Chloroform	U/Ex	Genetic activity = low. Exposed populations require further study. Investigate potential of protein adducts.
13. (N/A)	Chlordane	U/Ex	Review all criteria.
14. (N/A)	Nitropyrene	U	Review all criteria.
15. (N/A)	1,3-dichloropropene	U	Review all criteria.
16. (55)	Toluidine, O-	U/L	Genetic activity = low. Population questionable. Minimal adduct informtion. Investigate protein adduct formation.
17. (5)	Ethylene dichloride	U	More information is needed on all criteria. Could demonstrate a unique adduct.
18. (41)	Benzyl chloride	L/M, U/Ex	Genetic activity needs to be determined. Additional information is needed.
19. (40)	Dimethyl carbamoyl chloride	U/L	Genetic activity needs to be determined. Population data were questionable. Minimal suportive research data.
20. (N/A)	Malathion	U/L	Investigate protein adduct formation.

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Table 1. (concluded)

Number [a]	Chemical	Rating	Data Summary
21. (33)	Mephalan		Put aside for possible use in other studies.
22. (57)	Mitomycin C		" "
23. (38)	Thioacetamide		" "

The chemicals in Table 1 were rated on their genetic activity, adduct formation, exposed population availability, and research status. Definitions of the notation used are as follows:

[a] (#) The number in parentheses is the sequential number as it appeared in Appendix D.
(N/A) Indicates that the chemical is not listed in Appendix D.

[b] L Indicates a low priority ranking.
M Indicates a medium priority ranking.
H Indicates a high priority ranking.
U Indicates an unclassified rating. Assigned to chemicals for which the potential for study existed, but for which the information available at the time was inadequate to support any judgement..
Ex Indicates that further information is desired on exposure monitoring of protein adducts.

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The complete report, entitled "Selection of Adduct-Forming Chemicals for Human Monitoring Studies," (Order No. PB92-132877/AS; Cost: $19.00, subject to change) will be available only from:
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