United States
Environmental Protection
Agency

Environmental Monitoring
Systems Laboratory
Las Vegas, NV 89193-3478

Research and Development

EPA/600/S4-89/035 Feb.1990

EPA

Project Summary

Protein Adduct-Forming Chemicals for Exposure Monitoring: Chemicals Selected for Further Study

F. C. Schnell and T. C. Chiang

The present report is an expanded treatment of those chemicals recommended for further study by a previous U.S. EPA internal report entitled "Protein Adduct-Forming Chemicals For Exposure Monitoring: Literature Summary and Recommendations." The latter report summarized the literature regarding adducts formed by xenobiotics with proteins, particularly hemoglobin and serum albumin, and examined the feasibility of their use as dosimeters of exposure. Recommendations were made with respect to those compounds, protein adducts and detection methods best suited to monitoring human exposure to toxic chemicals, particularly those occurring at Superfund sites and others of interest to the EPA. Those recommendations were summarized in the form of a table entitled "Prioritized List of Protein Adduct-forming Compounds of Interest to the EPA."

The above mentioned, prioritized list of chemicals recommended for further study is reproduced in the introduction of the present report, where the ranking scheme is also explained. In subsequent sections of this report, the individual chemicals are discussed in the same order in which they appear on the prioritized list. The topics covered for each individual chemical are as follows: manufacture and use, sources and levels of exposure, known health effects, metabolic detoxification and activation, host factors, adduct characterization, rates of adduct second order rate constants), dose-response relationships, background adduct levels, methods of adduct detection, and research needs.

This Project Summary was developed by EPA's Environmental Monitoring Systems Laboratory, Las Vegas, NV, 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)

Introduction

The Environmental Protection Agency (EPA) is charged with protecting human health and the environment, and it has acted by placing restrictions and regulations on the use of chemicals that have been shown to be detrimental to human health or to the environment. Accurate dose measurements are critical to the evaluation of chemical-related health risks and to the subsequent development of realistic regulations to ameliorate those risks. Accordingly, the EPA has developed an initiative designed to develop, refine and apply appropriate biomarkers that can be used in conjunction with environmental monitoring data to provide a better estimate of exposure and risk for individuals and populations. Macromolecular adducts formed as a result of chemical exposure are among the biomarkers that the Agency, under its

Biomarker Initiative, is currently evaluating for use in future human biomonitoring studies.

A 1987 report entitled "Carcinogen-DNA Adducts: Introduction, Literature Summary and Recommendations" summarized the literature concerning adducts formed by xenobiotics with DNA and discussed the feasibility of their use as monitoring tools in exposure assessment. Current interest in this area is based on the knowledge that certain chemicals, including the majority of carcinogens and mutagens, are or may be metabolized to form reactive electrophilic species. These reactive electrophilic covalently bind to nucleophilic sites in cellular macromolecules such as DNA, RNA and various proteins. It was recognized that measurements of protein adducts, particularly those of hemoglobin and serum albumin, may provide a more quantitative measure of exposure over longer periods of time than is generally obtainable from measurements of DNA adducts.

A previous U.S. EPA internal report entitled "Protein Adduct-Forming Chemicals For Exposure Monitoring: Literature Summary and Recommendations" summarized the literature regarding adducts formed by xenobiotics with proteins, particularly hemoglobin and serum albumin, and examined the feasibility of their use as dosimeters of exposure. Recommendations were made with respect to those compounds, protein adducts and detection methods best suited to monitoring human exposure to toxic chemicals, particularly those occurring at Superfund sites and others of interest to the EPA. Those recommendations were summarized in the form of a table entitled " Prioritized List of Protein Adduct-forming Compounds of Interest to the EPA".

The selection criteria were designed to identify the most suitable protein adducts for use in monitoring human exposure to chemicals of interest to the EPA. Those criteria were as follows:

(1) The chemical should be identified as being of interest to the U.S. EPA.

(2) The chemical should form measurable amounts of a distinct, well characterized protein adduct.

(3) The background levels of that adduct in non-exposed populations should be as low as possible.

(4) The adduct must be readily measurable by existing analytical techniques.

(5) The adduct must be accumulated and eliminated in a predictable, dose-related manner.

(6) Both exposed and control populations must be available for a monitoring study of the adduct-forming chemical.

(7) Current levels of human exposure to the adduct-forming chemical should be associated with some potential, adverse health effect(s).

The present report is an expanded treatment of those chemicals recommended for further study by the above-mentioned, protein adducts summary report. The prioritized list of chemicals recommended for further study (Table 1) is reproduced in the introduction of the present report, where the ranking scheme is also explained.

In subsequent sections of this report, the individual chemicals are discussed in the same order in which they appear on the prioritized list. The topics covered for each individual chemical are as follows: manufacture and use, sources and levels of exposure, known health effects, metabolic detoxification and activation, host factors, adduct characterization, rates of adduct formation (i.e., second order rate constants), dose-response relationships, background adduct levels, methods of adduct detection, and research needs.

Conclusions and Recommendations

Group I Chemicals

Of the simple alkylating and arylating agents, ethylene oxide(EO), propylene oxide(PO) and styrene are recommended as having the greatest potential in human monitoring studies using protein adducts. EO, PO and styrene are all produced and used in the U.S. and rank high on the list of adduct-forming compounds of interest to the U.S. EPA. Also, one of the simpler existing methods of protein-adduct analysis, the modified Edman degradation procedure, is applicable to all three of these compounds.

EO has perhaps the largest research base of any protein adduct-forming chemical. Although large amounts of EO are produced and used by industry in the U.S., the highest exposures result from a relatively minor use of the chemical, i.e., the sterilization of hospital equipment. Because EO is distributed almost uniformly throughout the body and reacts directly with tissue macromolecules, its protein adducts more nearly reflect the level of DNA adducts in different ti than any other chemical studied thus

PO, a closely-related homologu EO, should exhibit similar pharm kinetics. The highest exposures tc are likely to occur among produ workers. The background levels of P adducts are lower than those of E adducts, making the former the informative biomarkers of exposure a larger range of exposure. Resul studies of occupational exposure t and PO suggest that hemoglobin a levels may be more sensitive to exposure to PO than EO.

Compared with ethylating agents simple arylating agent styrene proc adducts which exhibit lower backgr levels and a cleaner GC elution pr Both factors would enhance sensitivity with which styrene adc could be detected by chromatogra procedures. Investigators at Colu University are currently conducti study of occupational exposur styrene in boat-building facilities of N and Connecticut. The heaviest expos occur during hull and deck laminatic addition to hemoglobin adducts, study proposes to characterize validate four other biomarker exposure: lymphocyte DNA add micronuclei, sister chromatid excha and unscheduled DNA synthesis.

Human exposure to ethylene o propylene oxide and styrene can be monitored by measuring t hydroxyethyl-, hydroxypropyl- hydroxyphenylethyl-valine addu respectively. Background levels of valine adducts are lower than those o histidine and cysteine adducts, and be measured by a modified Ed degradation technique which elimin the need to perform tedious and t consuming analyses of total pro hydrolysates or enzymatic digests. U basic conditions in the presenc pentafluorophenyl isothiocyanate, modified terminal amino acids (valin hemoglobin) are selectively cleaved the protein. The resulting pe fluorophenyl-thiohydantoin (F5PTH) vatives may then be extracted analyzed by negative chemical ioniz mass spectrometry (NCIMS).

Because styrene, 7, 8, oxide, major reactive metabolite of styrene, a shorter biological half-life than ethy oxide, it is possible that more sty adducts are formed with albumin in liver cells than with hemoglobin in the cell. However, no reports of styr albumin adducts were found in literature. If styrene oxide does

Table 1. Prioritized List of Protein Adduct-Forming Compounds of Interest to the EPA

Group I - Simple Alkylating and Arylating Agents That Form N -Terminal Valine Adducts.
(1) Ethylene Oxide
(2) Propylene Oxide
(3) Styrene

Group II - Aromatic Amines That Form Hydrolyzable Cysteine Adducts.
(1) 4-Aminobiphenyl
(2) Benzidine
(3) MBOCA
(4) o-Toluidine

Group III - Chemicals That Form Hydrolyzable, but Less Well-Characterized Adducts.
(1) N-Nitrosonornicotine
(2) Benzo(a)pyrene
(3) 1 - Nitropyrene

Group IV - Chemicals That Form Characterized, but Non-Hydrolyzable Adducts.
(1) Vinyl Chloride
(2) Ethylene Dichloride
(3) Acrylonitrile
(4) Acrylamide
(5) Chloroform

Group V - Chemicals That Form Poorly Characterized Adducts.
(1) Benzene
(2) Formaldehyde
(3) 2,4-Toluene Diisocyanate
(4) 7,12 - Dimethylbenzanthracene
(5) Epichlorhydrin
(6) Benzyl Chloride
(7) Pentachlorophenol

dducts at the N-terminal aspartate of serum albumin, they might be detectable by the modified Edman procedure. However, no efforts to detect N-terminal aspartic acid adducts in serum albumin using the modified Edman procedure have been reported in the literature.

In a highly desirable alternative to GC-MS, the modified valine derivatives produced by the modified Edman degradation procedure might be detected using immunological techniques. If an antibody could be raised against the pentafluorophenyl-thiohydantoin (F_5PTH) derivative of alkylated N-terminal valine, a rapid, inexpensive method (e.g., a competitive ELISA) might be developed to monitor human exposure to ethylene oxide, propylene oxide and styrene on a large scale, one which could simultaneously be validated by GC-MS of the same analyte (i.e., the F_5PTH valine derivative). To cite a precedent, antibodies have been developed and used to detect benzo(a)pyrene tetrols released by acid treatment of benzo(a)pyrene-modified hemoglobin.

Group II Chemicals

Benzidine and 4-aminobiphenyl (4-BP) are established human bladder carcinogens, and benzidine and MBOCA rank high on the list of adduct-forming chemicals of interest to the U.S. EPA. The aromatic amines are also particularly attractive chemicals from the standpoint of exposure monitoring due to a remarkable situation that obtains with regard to their hemoglobin adducts. 4-ABP, benzidine, MBOCA and o-toluidine, among others, are all metabolized by a pathway that leads to the formation of large amounts of an acid-labile sulfinamide adduct of the β-93 cysteine of hemoglobin. The ultimate electrophile, a nitroso compound, is formed in the erythrocyte by co-oxidation of the N-hydroxylamine metabolite and hemoglobin. Under mildly acidic conditions, the sulfinamide bond is cleaved, regenerating the free amine which can then be extracted, derivatized and analyzed by GC-NCIMS. The development of antibodies to the acid-released material would make possible a rapid, inexpensive and extremely sensitive assay for the entire class of compounds.

None of the aromatic amines mentioned above is still made in the U.S., but benzidine (and possibly 4-ABP) is a metabolite of the widely used, benzidine-based dyes, while the other four all occur in cigarette smoke and have been measured in studies of smokers vs non-smokers. Also, measurable occupational exposure to 4-ABP may still occur in the form of an unwanted side product of other amines or dyes. MBOCA, a suspected bladder and liver carcinogen, is still widely used in the polyurethane industry, where an occupationally exposed population should be available. The research findings of investigators at Michigan State University which are relevant to hemoglobin-aromatic diamine adducts in general and MBOCA adducts in particular, are discussed in this report.

Any future study of human exposure to any carcinogenic aromatic amine should make a point of identifying the acetylator phenotype of the individual subjects because so-called "slow acetylators" are known to have a higher risk of bladder cancer than so-called "fast acetylators." This report includes a discussion of the possibilities of simultaneously monitoring levels of two different protein adducts of an aromatic amine, i.e., one acetylated and one non-acetylated, in an effort to clarify the relationship between acetylator status and effective exposure to aromatic amines.

Group III Chemicals

N-nitrosonornicotine (NNN), benzo (a) pyrene (B (a) P), and l-nitropyrene (1-NOP), like the aforementioned aromatic amines, form Hb adducts the cleavage products of which may be released into solution by treatment with dilute acid or base. However, these hydrolyzable Hb adducts, unlike those of aromatic amines, are formed in only small amounts and are not as well characterized (e.g., the parent adduct of the hydrolysis product may not be known.)

The major tobacco alkaloid nicotine is the precursor to the tobacco-specific nitrosamines-NNN and 4-(methylnitrosamino)-l-(3-pyridyl) -l-butanone (NNK). The latter are among the most important carcinogens in cigarette smoke and are the major carcinogens present in so-called "smokeless" tobacco. Both compounds may be activated *via* α-hydroxylation (the major activation pathway) to methylating agents or, to a lesser extent, to 4-(3-pyridyl)-4-oxobutyldiazohydroxide which forms unique, bulky adducts in both Hb and liver DNA in rats. The latter adducts are detectable as their hydrolysis product 4-hydroxy-l-(3-pyridyl)-1-butanone (HPB).

The likely relevance of the HPB-yielding adduct to carcinogenic risk, and the absence of confounding sources,-make the tobacco-specific nitrosamines NNN and NNK potentially useful model compounds for human monitoring studies. To date, however, the scientific literature contains no report of NNN/NNK-Hb adducts having been detected in humans. Because methyl adducts cannot be easily attributed to any specific exposure, efforts to detect NNN/NNK adducts in humans should focus on the HPB-yielding adduct. The work of Stephen Hecht of the Naylor Dana Institute should be followed closely in this regard. If HPB-yielding adducts are detectable in blood samples of exposed humans, low adduct levels and high inter-individual variation can be anticipated.

Benzo(a)pyrene, a carcinogenic polycyclic aromatic hydrocarbon (PAH), is ubiquitous in the environment, even though it is not commercially produced in the U.S. The benzo(a)pyrene diol epoxide (BPDE) metabolite thought to be the ultimate carcinogen binds to Hb *in vivo*. Treatment of the BPDE-modified Hb with dilute acid releases a minor, acid-labile adduct into solution as the corresponding BPDE tetrol. The acid-released tetrols may then be analyzed using HPLC-fluorescence spectroscopy.

However, the vast majority of B(a)P-globin adducts are relatively stable to acid, and some effort should be made to characterize these adducts, some of which may possibly be better biomarkers of B(a)P exposure than the tetrol-releasing adducts. Intact B(a)P-Hb adducts have been analyzed by laser-induced fluorimetry and by competitive ELISA. In both cases, Hb was enzymatically digested prior to analysis to expose the intact adducts and samples were enriched in B(a)P-modified peptides by elution from an immunoaffinity column.

The highest level of B(a)P adducts occurs in liver proteins, of which newly synthesized albumin is one, and total binding of radiolabeled B(a)P is reportedly much higher in serum albumin than in Hb. An investigation of B(a)P-serum albumin adducts is therefore recommended. Serum albumin adducts have proven very useful for monitoring exposure to aflatoxin B_1,another compound that binds poorly to Hb.

1-Nitropyrene, a widespread environmental PAH, is neither produced nor used commercially in the U.S. Exposure to this carcinogenic PAH occurs via inhalation of airborne particulates, diesel emissions, coal fly ash, carbon black photocopier toners and smoke from nitrate-fortified cigarettes. The formation of a single major Hb adduct is linearly related to the oral dose of 1-nitropyrene in rats. The acid-labile Hb adduct, which is released into solution during the precipitation of globin in acidic acetone, has been tentatively identified as the cysteinyl sulfinamide of a ring-hydroxylated N-hydroxylamine meta-bolite. This adduct may be useful for monitoring human exposure to 1-nitropyrene. However, no studies have yet been done in humans, and the adduct needs to be further characterized in animals.

Group IV Chemicals

Vinyl chloride (VC), the most important of the industrial vinyl monomers, is heavily used in the plastics industry for the manufacture of polyvinyl chloride and copolymers. While the potentially exposed population is probably quite large, actual levels of exposure to VC in the workplace are probably quite low (i.e., below the 1 ppm, 8 hr time wieghted average (TWA) standard promulgated by OSHA in 1983), because VC is an established human carcinogen. In fact, hepatic angiosarcoma, a rare form of neoplasia, occurs almost exclusively among VC reactor cleaners. A protein adduct-based method for expos monitoring would be particularly usef the case of vinyl chloride, since reliable method currently exists for r itoring exposure to VC at concentrat below 5 ppm

The reactive metabolites of VC chloroethylene oxide (CEO) and chloroacetaldehyde (CAA), introduce oxoethyl groups at the sulfhydryl gr of cysteine, the 1-N and 3-N positior histidine, and the amino nitrogen o terminal valine. The acid-stable add S-(2-oxoethyl)cysteine and N-(2-oxoe histidine are currently of limited use monitoring VC exposure in humans. N-terminal valine adduct should h more potential as a biomarker exposure,but the modified Edr degradation procedure has, appare not yet been applied to the study of protein adducts, nor have VC-se albumin adducts been investigate might prove worthwhile,therefore examine serum albumin as well as using the modified Edman degrada method.

Ethylene dichloride (EDC), the lar volume chlorinated organic compc currently produced in the U.S., is u primarily in the manufacture of v chloride. EDC and VC are metabolize much the same way; the epo metabolites of both produce 2-oxoe adducts which, when hydrolyzed analysis, are reduced to 2-hydroxye adducts. Thus, the protein adducts of EDC and EO (which yields 2-H adducts directly) cannot easily distinguished from one another i protein hydrolysate. As with VC, modified Edman procedure has not been used to assay levels of the oxoethylvaline adduct. The glutath (GSH) conjugate of EDC also form unique, bulky DNA adduct, S-[2-guanyl)ethyl]GSH, which, measured urinary metabolite, might serve as a n specific biomarker for EDC exposure related compound, ethylene dibrom has been shown to bind to se albumin (139 nmol/g) in treated rats.

Acrylonitrile (ACN) is an impor industrial monomer used extensivel the manufacture of synthetic fib rubbers and resins for a variety consumer goods. It is also usec produce acrylamide (AN), an indus compound used in the manufactur polymers for water treatment. I chemicals are neurotoxic in mam and carcinogenic in rodents, exposures are expected to be kept minimum. Animal data (the only available) indicate that both of th

direct-acting agents are detoxified primarily by conjugation with GSH, and both are direct-acting agents that may also form protein adducts via their epoxide metabolites. When modified Hb is hydrolyzed for analysis, both the nitrile group of acrylonitrile and the amide group of acrylamide are transformed into carboxyl groups. Thus, the adduct formed by direct Michael addition of either compound to cysteine will be analyzed as S-(2-carboxyethyl)cysteine (the major adduct), while the adduct formed by reaction of the epoxide metabolite of either chemical with cysteine will be analyzed as S-(2-carboxy-2-hydroxyethyl) cysteine (a minor adduct).

The consequence for biological monitoring is that acrylamide and acrylonitrile exposures cannot be distinguished from one another using the hydrolysis products of their cysteine adducts. However, if both glycidamide and glycidonitrile form N-terminal valine adducts, the milder pH changes involved in the modified Edman degradation procedure might leave the amide and nitrile groups of the respective adducts intact. If this approach were successful, then the glycidamide-and glycidonitrile-valine adducts might be separated and analyzed by GC-MS (provided the appropriate column were used) or by LC-MS. If ACN/AN-adducts of N-terminal valine cannot be detected in Hb of exposed animals, then ACN/AN-adducts of N-terminal aspartate should be sought in serum albumin.

Chloroform is produced and used in the U.S. principally for use as a reactant in the manufacture of freon (fluorocarbon-22) and fluorocarbon plastics, and as an extractant and industrial solvent in the dye and drug industries. Production workers make up the best exposed population, but again, exposures are expected to be low. Although chloroform is not mutagenic in bacteria, it causes cancer in rodents, and is classified by the U.S. EPA as a probable human carcinogen.

In vivo binding of radiolabeled chloroform to rat Hb increases linearly over a wide range of dose and is eliminated at a rate consistent with the lifespan of erythrocytes. *In vitro* the reactive metabolite of chloroform, phosgene, forms an unusual cyclic 2-oxothiazolidine derivative with cysteine in Hb. This adduct is hydrolyzed during preparation for GC-MS analysis and measured as N-hydroxymethyl cysteine. However, it should be noted that there is some controversy over the identification of N-hydroxymethyl cysteine as the major product by GC-MS, because the hydroxymethylamino moiety should have been too unstable to be isolated as such.

In view of the unreliability of blood levels and breath levels of chloroform for monitoring exposure, a protein adduct-based method would be particularly useful. However, no studies of the formation of chloroform adducts in human Hb have yet appeared in the literature. More research is needed to identify the chloroform-protein adducts formed *in vivo*.

Group V Chemicals

Benzene is produced in great quantity (1.72 billion gallons in 1988) in the U.S. where it is widely used as a chemical intermediate in the synthetics industry. Because it is produced by natural as well as manmade sources, benzene is ubiquitous in the environment. The largest number of people are exposed to benzene by inhaling automobile exhaust and cigarette smoke. An established bone marrow toxin, benzene has been associated with myelogenous leukemia in man, and the U.S. EPA classifies benzene as a Class A human carcinogen. Hence, the Agency's interest in finding improved methods of monitoring exposure to this chemical is well-placed.

However, more research on benzene-protein adducts is needed in all areas, from adduct identification to method development, before the question of the feasibility of using protein-adducts to monitor human exposure to benzene can be adequately addressed. In particular, efforts should be made to identify and characterize an N -terminal valine adduct of benzene in Hb of animals and humans. It is recommended that work on benzene-Hb adducts at the Lovelace Inhalation Toxicology Research Institute be followed closely.

Formaldehyde is of interest to the U.S. EPA because of (1) its high level of production (2.81 million tons in 1985) and widespread use (e.g., in particle board, plywood and ureaformaldehyde insulation), (2) the opportunity for exposure in the general population (e.g., in mobile homes and remodeled offices), and especially (3) the report that high doses cause nasal cancer in rats. However, the induction of nasal cancer in rats by 6-15 ppm formaldehyde--4-5 ppm is intolerable to most humans--does not appear to be particularly relevant to the human situation. In any case, it is not presently feasible to monitor formaldehyde exposure using protein adducts, because no stable formaldehyde-protein adducts have been described in the literature. The DNA protein cross-links that are formed by formaldehyde are either unstable or rapidly repaired.

2-4-Toluene diisocyanate is a highly reactive chemical used in the manufacture of rigid polyurethane products. Because it is such a potent allergen, OSHA has set its TLV at 5 ppb (parts per billion), 8 hr-TWA, and 20 ppb for 10 minutes. TDI also causes cancer in rats and female (but not male) mice when administered by gavage. Although the isocyanate functional groups of TDI are reported to react extensively with -OH, -SH, or -NH groups on proteins, no specific TDI-protein adducts appear to have been described in the literature. Instead, TDI exposure has been monitored with variable success by measuring the titer of antibodies to endogeneous adducts in sera of exposed persons. Given the efficiency with which TDI apparently reacts with protein, it should not be difficult to chemically identify one or more TDI-protein adducts. Of special interest for the purpose of monitoring would be any adducts formed at the N-terminal amino acids of either Hb or serum albumin.

7,12-Dimethylbenzanthracene (DMBA) is an extremely potent animal carcinogen that is often used as a positive control in carcinogenicity assays. DMBA is not produced commercially, and its only use is as a research chemical. Consequently, there is no exposed population for study. The sulfate ester of a major microsomal metabolite of DMBA, 7-hydroxy-12-methyl-benz[a] anthracene (HMBA), covalently binds to cysteine, lysine and methionine residues in protein *-in vitro* However, no *in vivo* studies of DMBA-protein adducts were found in the literature.

For epichlorhydrin, benzyl chloride and pentachlorophenol, little information is available apart from the fact that the radiolabeled compounds do bind to amino acids or protein *in vitro*. No *in vivo* studies have been performed, and no specific protein adducts have been identified.

Future Directions

No efforts appear to have been made to use the modified Edman procedure to detect N-terminal aspartic acid adducts in serum albumin. The reactivity of the amino groups of valine and aspartic acid should be quite similar. The real question is whether the carboxyl group of aspartate will compete with the amino group for either the adduct-forming

species or the Edman reagent. If the method could be demonstrated to work with serum albumin as well as with Hb, then the modified Edman procedure might be useful in monitoring exposure to certain chemicals that react poorly with Hb.

Although N-terminal alkylvalines and cysteine sulfinamides can be detected and quantified by methods that eliminate most of the time-consuming steps associated with protein analysis, thorough extraction and derivatization of each blood sample is still required. Also, GC-MS is not ideally suited to mass screening due to the expense of the instrumentation and the level of training January 5, 1990 12:24 PM required for itsoperation. The obvious solution to these problems is the development of antibodies for the modified valine residues and the free aromatic amines generated by the procedures described above. Such antibodies could be used to quantify hydrolyzable adducts in the super-natantafter the adducts had been released from the protein and concentrated into a smaller volume.

The use of antibodies that could recognize non-hydrolyzable adducts *in situ* would cut out even more steps in the analysis. While some such adducts will occur on the surface of the prot molecule, others will be buried hydrophobic clefts and be le accessible to the antibody. Neverthele it should be possible to expose most not all, hemoglobin adducts by treati the sample with denaturing agents proteases prior to analysis. Mc research is required to determi whether antibodies to protein adducts c be developed which can be used quantify adduct levels in intact prote without severely reducing the sensitiv of the immunoassay.

F.C. Schnell and T.C. Chiang are with Lockheed Engineering and Sciences Co., Las Vegas, NV 89119
***Charles H. Nauman** is the EPA Project Officer (see below).*
The complete report, entitled "Protein Adduct-Forming Chemicals for Exposure Monitoring: Chemicals Selected for Further Study," (Order No. PB90 151762/AS; Cost: $23.00, subject to change) will be available only from:
National Technical Information Service
5285 Port Royal Road
Springfield, VA 22161
Telephone: 703-487-4650
The EPA Project Officer can be contacted at:
Environmental Monitoring Systems Laboratory
U.S. Environmental Protection Agency
Las Vegas, NV 89193-3478

United States
Environmental Protection
Agency

Center for Environmental Research
Information
Cincinnati OH 45268

Official Business
Penalty for Private Use $300

EPA/600/S4-89/035