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ENVIRONMENTAL SCIENCES DIVISION

BIOLOGICAL (MOLECULAR AND CELLULAR) MARKERS OF TOXICITY

Semi-annual Technical Progress Report (No. 3)
October 1, 1989 to March 31, 1990

John F. McCarthy

Date Published - April 1, 1990

Prepared for

U.S. Army Biomedical & Development Laboratories
Fort Detrick, MD 21701-5010

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Oak Ridge, Tennessee 37831-6258

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Contract No.:

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Interagency Agreement no. 1016-B047-A1

Contract Title:

BIOLOGICAL (MOLECULAR AND CELLULAR) MARKERS OF TOXICITY

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Semi-annual Technical Progress Report (no. 3)
October 1, 1989 to March 31, 1990

Contracting Officer's Technical Representative:

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Fort Detrick, MD 21701-5010

I. PURPOSE AND SCOPE OF RESEARCH EFFORT

The overall objective of this study is to evaluate the use of the small aquarium fish, Japanese Medaka (Oryzias latipes), as a predictor of potential genotoxicity following exposure to carcinogens. This will be accomplished by quantitatively investigating the early molecular events associated with genotoxicity of various tissues of Medaka subsequent to exposure of the organism to several known carcinogens, such as diethylnitrosamine (DEN) and benzo[a]pyrene (BaP).

The Japanese medaka has recently been used as a bioassay model to screen for carcinogenic compounds in water. The primary endpoint for most small fish carcinogenesis studies is the histopathogenic identification of a neoplastic lesion. Such lesions occur mainly in the liver, although other tissues in which lesions have been observed include nervous tissue, kidney, mesenchymal tissue, skeletal and smooth muscle. The histogenesis of liver neoplasms in fish is similar to that in rodents. Following exposure to chemical carcinogens, preneoplastic lesions appear as eosinophilic foci, basophilic foci, followed by tumor formation (Aquatic Toxicol. 11:113-128;1988). This is identical to the process that appears to occur in rodents and other mammals (Environ. Health Perspec. 75:65070;1987).

Therefore, if we are to understand in any depth the processes by which environmental chemicals exhibit genotoxicity, new and innovative techniques will be needed that elucidate the fundamental cellular nature of the various steps subsequent to exposure to these agents.

Because of the often long latent period between initial contact with certain chemical and physical agents in our environment and subsequent expression of deleterious health or ecological impact, the development of sensitive methods for detecting and estimating early exposure is needed so that necessary interventions can ensue. A promising biological endpoint for detecting early exposure to damaging chemicals is the interaction of these compounds with cellular macromolecules such as Deoxyribonucleic acids (DNA). This biological endpoint assumes significance because it can be one of the critical early events leading eventually to adverse effects (neoplasia) in the exposed organism.

The research will proceed along several interrelated lines of investigation, which have recently been reevaluated and revised:

Task 1. Evaluate current analytical techniques and

methodologies for their effectiveness to detect and quantitate biological responses at the molecular level to the action of genotoxic agents. Particular emphasis will be placed on of the adaptation of these methods to the detection of enzyme activities associated with detoxication and damaging events that occur to the DNA in the liver;

Task 2. Develop protocols for the exposure of Medaka under controlled laboratory conditions to DEN and BaP and investigate the effects these chemical carcinogens have on the suite of molecular markers selected;

Task 3. Evaluate the short-term responses of the molecular markers as suitable biomarkers to estimate exposure and predict cellular effects to genotoxic chemicals.

The work proposed here focuses on the more fundamental aspects of the problems that deal with the detection of exposure to genotoxic agents through the appropriate use of molecular markers and the potential to predict subsequent adverse effects. Many chemical carcinogens and mutagens have the capacity to cause various types of DNA damage as a result of the interaction of highly reactive metabolites with DNA. Such interactions can form stable adducts with DNA, adducts which result in the formation of alkali-labile apurinic sites, and unstable adducts which cause strand breaks (American Sci. 70:386-393;1982). Indirectly, the fidelity of post replicative modification of DNA (such as minor nucleotide composition) can be affected by genotoxic agents. Each type of damage to the integrity of the DNA, if left uncorrected, could trigger a sequence of events that culminates in the appearance of an overt malignancy. Estimates of these various types of damage to DNA, along with an appraisal of the organism capability to maintain the integrity of it's DNA (repair), will provide the basis for determining bioavailability of the genotoxic agent and for estimating exposure. In addition the information may be used as a short-term predictor of the potential for long-term deleterious effects.

II. OVERALL PROGRESS

1. Task 1.

The purpose of this task is to evaluate the feasibility of using currently existing analytical techniques to detect molecular markers of genotoxicity, and included: (a) enzymes of detoxication and, (b) changes to the integrity of DNA. This task has essentially been completed and was reported in detail in Semi-Annual Technical Progress Report No.1 (ORNL/M-829, dated April 1, 1989). With respect to detoxication enzymes in the medaka, methodologies for the measurement of 7-ethoxyresorufin O-

deethylase (EROD) and Glutathione S-transferase (GST) were successfully applied. Also, existing methods and techniques for the assessment of DNA integrity (i.e., strand breaks, adduct formation, and minor nucleoside composition) were adapted.

2. Task 2.

The purpose of this task is to evaluate the effect of exposure of the Japanese medaka to known genotoxic agents on the response of a suite of molecular markers. This complex task has been subdivided into several subtasks and the progress on each is reported separately.

a. Protocols for exposure.

A protocol for exposure to DEN was reported in detail in Semi-Annual Technical Progress Report No.1 (ORNL/M-829, dated April 1, 1989). Molecular marker responses observed in Japanese medaka exposed to DEN employing this protocol have been examined (see Task 2.b. below, and Appendix A).

The following protocol for exposure to BaP was developed (see Task 2.c. below):

> Acclimate 60 adult Japanese medaka (Oryzias latipes) (approximately three months of age-60% female/40% male) in a 30-L aquarium at 25° for two weeks. Aerate the water continuously and renew 25% of the volume every two days. Provide food ad libitum and maintain light on a 16 hour on and 8 hour off schedule. At the end of the acclimation period, add BaP (in tween 80) to a concentration of approximately 10 ug/L (Environ. Toxicol. Chem. 8:863-869;1989). Continue regular maintenance for sixteen days, and renew 50% of volume of aquarium ever two days with water containing 10 ug/L of BaP (in tween 80). Control groups are medaka similarly treated but exposed to tween 80 (BaP vehicle) only, and medaka similarly treated, but without exposure. Remove ten fish (6 female/4 male) from each group at days zero, one, two, four, eight and sixteen. Sacrifice the animals by cervical snip and remove the following tissues: liver, gall bladder, gill, testes and ovaries. Store all tissues, including carcass, not to be used immediately in liquid N.

b. Exposure of medaka to DEN.

This subtask has been completed and results reported in detail: (a) at the Annual Carcinogenicity Research Review Meeting (USABRDL, Fort Detrick, MD, August 8-9, 1989); (b) in the Semi-Annual Technical Progress Report No.2 (ORNL/M-948, dated October 1, 1989); (c) at the "Animal-to-Human Extrapolation Symposium"

(San Antonio, TX, March 13-15, 1990); and (d) as a manuscript submitted for publication to Neuroscience and Biobehavioral Reviews (see Appendix A of this report).

c. Exposure of medaka to BaP.

Medaka have been exposed to BaP according to the protocol reported in Task 2.a. above. Tissues recovered are in the process of being analyzed for the suite of molecular markers detailed in previous Technical Progress Reports. It is anticipated that a preliminary report of these results will be available and forwarded by June 1, 1990. A final, detailed report will be made at the Annual Carcinogenicity Research Review Meeting in August of this year, and will be a part of the next Semi-Annual Technical Progress Report (No.4).

d. Application of new techniques & methodologies.

Flow cytometric analysis for the determination of DNA distribution in hepatocytes (Bull. Environ. Contam. Toxicol. 40:343-349;1988) has been implemented into the suite of molecular markers (see Semi-Annual Technical Progress Report No.2 for details).

Currently under consideration and evaluation are techniques to detect and measure: (a) DNA repair; (b) DNA damage due to free radicals; and (c) induction of stress proteins. It is anticipated that data on the occurrence of stress proteins after exposure of medaka to BaP will be incorporated into the results from that study.

3. Task 3.

The purpose of this task is to evaluate the short-term responses of the suite of molecular markers as suitable biomarkers to estimate exposure and predict cellular effects to genotoxic chemicals. It is anticipated that this study will provide data to demonstrate the usefulness of the Medaka as a bioassay model to evaluate the potential of environmental genotoxicity. The responses to be studied are important because they have been selected on the basis of existing experimental observations (i.e., they are key ingredients that provide evidence that exposure to genotoxic agents is resulting in toxicological interactions) and they can be used to make positive predictions.

III. PROBLEM AREAS

As pointed out in Semi-Annual Technical Progress Report No.2, one of the biggest problems we have encountered is

obtaining sufficient numbers of adult Medaka for experimental work. Because of the small size of the Medaka, and therefore the amount of tissue available for analyses, we must use adult animals. In order to address this problem, we started grow-out facilities at the Oak Ridge National Laboratory for rearing Medaka fry to adulthood. Medaka fry have been supplied by Dr. William Walker of the Gulf Coast Research Laboratory, Ocean Springs, MS. This grow-out facility provided animals of the size, and quantity needed for the initial BaP-exposure experimentation (see Task 2.c. above). This facility is critical for continued productive research on this project, but does extract a price in terms of time and labor needed to maintain the animals. Mainly because of this constraint, the Tasks of this project has been reevaluated and revised to ensure the timely completion of the work detailed without impacting budgetary requirements.

IV. WORK TO BE PERFORMED DURING THE NEXT SIX MONTHS

At present, approximately 1000 adult mekaka are available for experimentation with sufficient fry being maintained to supplement those used for this purpose.

Studies will continue to focus on alterations to DNA integrity and changes in detoxication systems of medaka exposed to BaP, however, it is anticipated that exposure protocols will be modified to include mixtures of potential genotoxic agents.

As mentioned in Task 2.d. above, the evaluation of new techniques to detect and measure DNA repair and DNA damage due to free radicals is in progress.

As data generated from the BaP exposure experiment becomes available, it will be compared to similar data generated from the DEN exposure experiment in an effort to evaluate the usefulness and effectiveness of the medaka bioassay model to predict exposure to genotoxic chemicals.

V. ADMINISTRATION COMMENTS

This report was prepared according to the instructions found in the U.S. Army Medical Bioengineering Research & Development Laboratory document entitled "Contractor Reporting Requirements for the Health Effects Research Division" dated march 1984.

VI. GANTT CHART

Attached.

VII. COST SUMMARY REPORT

1. PROJECT COST SUMMARY

Attached.

2. COST SUMMARY GRAPH

Attached.

VIII. APPENDIX "A"

1. Manuscript submitted for publication in Neuroscience & Biobehavioral Reviews.

Attached.

J.F. McCarthy, April 1, 1990

PROJECT COST SUMMARY

Project Title No.: Biological (Molecular and Cellular) Markers of Toxicity/U.S. Army 88PP8861
Organization/PI: Oak Ridge National Laboratory/J.F. McCarthy
Reporting Period: October 1, 1989 to March 31, 1990

Total Allocation: $ 6,982 Carry-over from 1st year
$160,000 Allocated 2nd year
$166.982

	Monthly Expenditures			Cumulative Expenditures			Available
	Budget	Actual	Variance	Budget	Actual	Variance	Balance
							166,982
Oct	13,915	7,507	-6,408	13,915	7,507	- 6,408	159,475
Nov	13,915	8,009	-5,906	27,830	15,516	-12,314	151,466
Dec	13,915	8,664	-5,251	41,745	24,180	-17,565	142,802
Jan	13,915	15,536	+1,620	55,660	39,716	-15,944	127,266
Feb	13,915	20,468	+6,553	69,575	60,184	- 9,391	106,798
Mar	13,915	26,288	+12,373	83,490	86,472	+ 2,982	80,510
Apr	13,915			97,405			
May	13,915			111,320			
Jun	13,915			125,235			
Jul	13,915			139,150			
Aug	13,915			153,065			
Sep	13,917			166,982			

Note: Cost data as of end of each month.
This report was prepared 4/1/90.

ORNL-DWG 89M-9499

Project Title: Biological (Molecular and Cellular) Markers of Toxicity

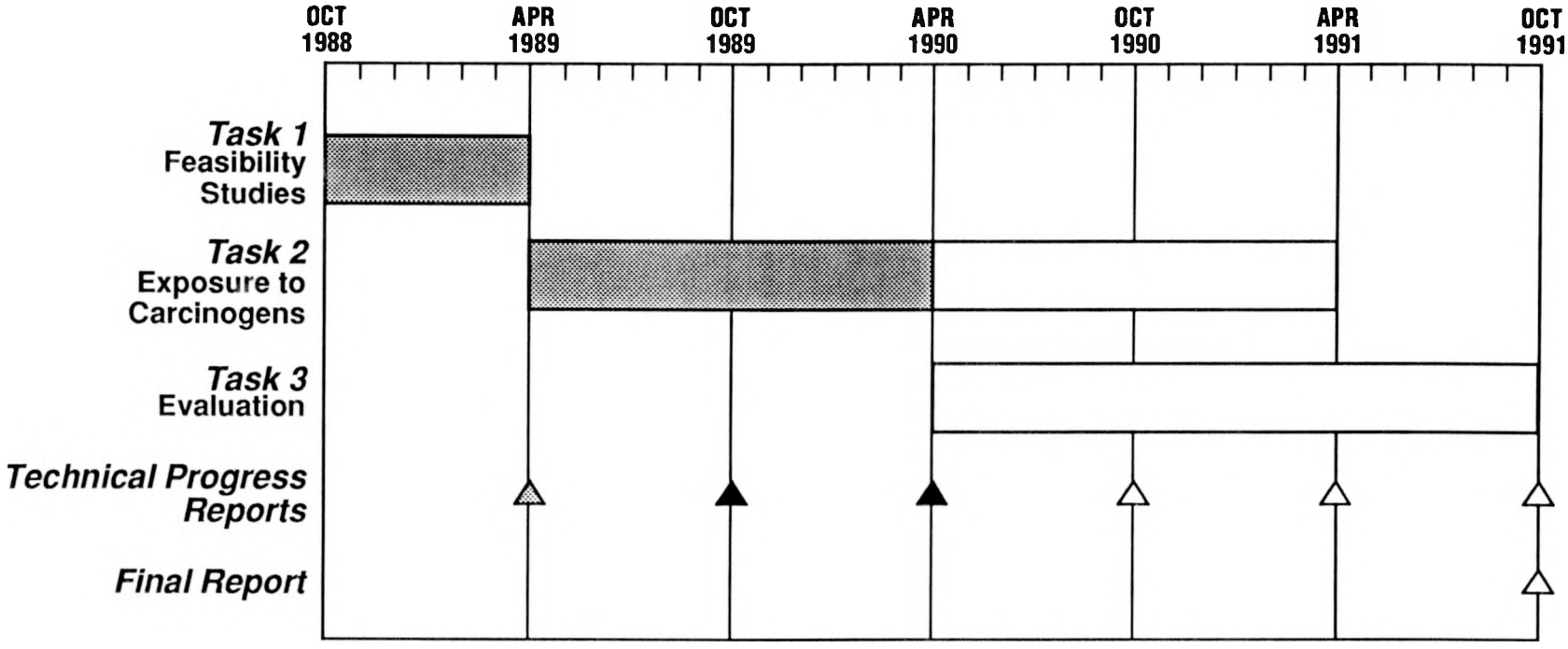
Contract No.: U.S. Army 88PP8861
IAG 1016-B047-A1

Reporting Period: October 1, 1989 to March 31, 1990

Performing Organization: Oak Ridge National Laboratory

Principal Investigator: J. F. McCarthy

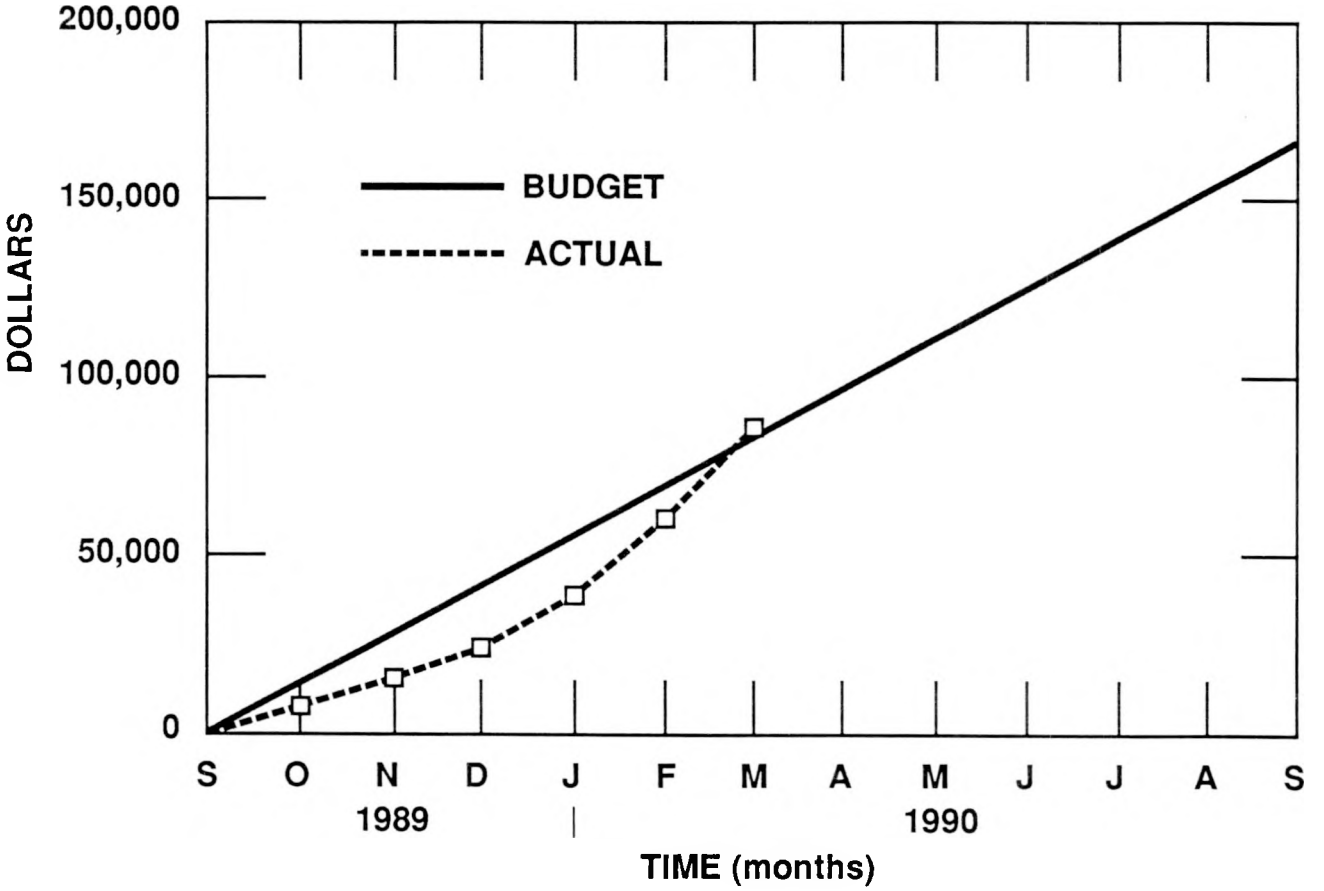
Date: April 1, 1990



ORNL-DWG 89M-9487

Cost Summary Graph

Project Title / No.: **Biological (Molecular and Cellular) Markers of Toxicity / U.S. Army No. 88PP8861**
Organization / PI: **Oak Ridge National Lab / J. F. McCarthy**
Reporting Period: **October 1, 1989 to March 31, 1990**
Total Allocation: **$166,982**



APPENDEX "A"

Manuscript submitted for publication in Neuroscience & Biobehavioral Reviews

Submitted for publication in:

Neuroscience & Biobehavioral Reviews

Title:

DNA ALTERATIONS AND ENZYME ACTIVITIES IN JAPANESE MEDAKA (Oryzias latipes) EXPOSED TO DIETHYLNITROSAMINE

Authors:

John F. McCarthy[1], Hank Gardner[2], Marilyn J. Wolfe[3] and Lee R. Shugart[1].

Addresses:

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Running head:

GENOTOXICITY IN JAPANESE MEDAKA

ABSTRACT

McCarthy, J.F., H. Gardner, M. J.Wolfe, and L. R. Shugart. DNA alterations and enzyme activities in Japanese medaka (Oryzias latipes) exposed to diethylnitrosamine. Several molecular and biochemical markers of genotoxicity have been adapted for measurement in the Medaka, and have been applied to describe the effects of treatment of the organism with diethylnitrosamine (DEN). DEN treatment inhibited Phase I detoxication enzyme activity and increased Phase II activity; a pattern of response that has been described in preneoplastic rodent cells. No O^6-ethyl guanine adducts were detected, and only slight, but statistically significant, increase in DNA strand breaks was observed. These results are consistent with a hypothesis that the prolonged exposure to the high levels of DEN employed, induced alkyltransferase activity which enzymatically removed any O^6-ethyl guanine adducts that formed but did not result in strand breaks or hypomethylation of the DNA such as might be expected from excision repair of chemically modified DNA.

Indexing terms:

Medaka; genotoxicity; DNA alterations; detoxifying activities; DNA strand breaks; 5'-methyldeoxycytidine; flow cytometery

INTRODUCTION

The research reported her was undertaken as an initial study to evaluate the suitability of the Japanese Medaka as a model organism for prediction of potential genotoxic effects from exposure to environmental contaminants. Fundamental understanding of the molecular and biochemical events that occur subsequent to exposure may make it possible to employ these responses as biomarkers that estimate exposure and predict cellular effects of genotoxic chemicals (9,11). Thus, it may be possible to determine if an individual chemical, or waste effluent poses a health threat based on exposure of Medaka to contaminant for the short time required for the biomarker to respond, rather than for the six months or more required to evaluate a histopathological effect. More fundamentally, critical evaluation of the genotoxic process at the molecular and cellular levels in Medaka may provide a foundation for using this convenient and cost-effective animal model for more directly extrapolating to human health effects, based on pharmacodynamic models. Before this approach can be accepted, it is necessary to demonstrate that molecular, biochemical and cellular events associated with genotoxicity are detectable in fish.

MATERIAL AND METHODS

1. Exposure Protocol.

Approximately 70 adult Japanese Medaka (Oryzias latipes) of both sexes were exposed at 25° to a solution of 200 mg/L of diethylnitrosamine (DEN) for 24 hours, followed by transfer to clean water for 6 days. This exposure protocol was repeated

three additional times. A control population was similarly treated, but without exposure to DEN. Ten fish in both groups were pooled (approximately equal numbers of each sex in each replicate) and livers removed. All analyses were performed on pooled liver tissue or, as noted, on whole individual animal minus liver (termed "carcass").

2. DNA Alterations.

a. DNA Strand Breaks - Stand breakage was measured in DNA isolated from the carcass by an alkaline unwinding assay as modified by Shugart (7). The technique is based on the time-dependent partial alkaline unwinding of DNA followed by determination of the duplex:total DNA ration (F value).

b. 5-Methyl Deoxycytidine Content (5m-dCyd) - Deoxyribonucleoside analysis was performed on DNA isolated from the carcass according to the procedure of Shugart (8).

c. DNA Adducts (Monoclonal Antibodies) - Analysis for O^6-Ethyl guanine adducts in liver DNA was by a noncompetitive solid-phase immunoassay (1).

d. Abnormal DNA Distribution of Hepatocytes (Flow Cytometry) - Liver tissue was prepared for flow cytometric analysis according to the procedure of McBee and Bickham (5) and frequency of DNA distribution within the hepatocytes was obtained.

3. Detoxication Enzymes.

a. Phase I Activity (Ethoxyresorufin-O-Deethylase, EROD) - EROD activity from the low speed supernatant of liver homogenate was measured fluorometrically at 30° (2).

b. Phase II Activity (Glutathione-S-Transferase, GST) - GST activity from the low speed supernatant of liver homogenate was determined according to the method of Habig (4).

4. Histology.

Four control and eight DEN-exposed animals were anaesthetized, sacrificed, fixed in Bouin's solution and Hematoxylin and eosin slides ere prepared for histopathological analysis. Animals were sectioned such that longitudinal sections were made of the entire body minus the caudal fin. Step sections were cut to obtain two paramedian sections each from the right and left sides of the fish and one mid-sagittal section. Two adjacent sections from each of these five planes were mounted for a total of ten sections per fish. This method of sectioning ensures that all major organs of the fish are represented on the slides.

RESULTS AND DISCUSSION

1. DNA Alterations.

Since alteration of the DNA is understood to be the initiating event in carcinogenesis (11), the effect of DEN on several types of DNA damage was evaluated in the Medaka, including early events such as adduct formation and secondary modifications of DNA integrity, as well as later, generally irreversible effects such as cytogenetic aberrations (5).

a. DNA Strand Breaks - Strand breaks were elevated in DNA of Medaka exposed to DEN (Table 1). Based on the observed F values (7), the DEN-exposed animals had 70% more strand breaks in their DNA than the non-exposed group. This is a relatively small difference in DNA integrity, compared to fish collected from contaminated streams (10) or exposed to low (1 ug/L) concentrations of BaP (7), in which levels in excess of 600% are found.

b. 5m-dCyt Content - The only methylated deoxynucleoside in eukaryotic DNA is 5m-dCyd. It has been demonstrated in cell

culture that chemical carcinogens and mutagens alter the normal patterns of DNA methylation by interfering with the fidelity of the normal post-replicative modification of the DNA. The hypomethylation of the DNA has been shown to lead to inheritable abnormalities in gene expression (3). The effect of carcinogens on DNA methylation patterns has been demonstrated in vivo in fish. Bluegill sunfish exposed to BaP (1 ug/L) for 40 d experienced a 50% decline in 5m-dCyd content in their DNA (8).

Based on a limited number of analyses, there does not appear to be any difference in exposed versus control fish (Table 1).

c. DEN-DNA Adducts - Three replicate samples of liver DNA from the DEN-exposed animals and four samples from the control animals were assayed, and no O^6-ethyl guanine were detected in any of the samples (detection limits are estimated to be approximately 1 adduct per 10^6 to 10^7 nucleotides). It is perhaps significant to note that this adduct can be enzymatically cleaved (dealkylated without concomitant breakage of DNA strands) by the activity of the enzyme, O^6-alkylguanine-DNA alkylase, and that malignant transformation may be associated with activation of this enzyme.

d. DNA Distribution in Hepatocytes - A typical result of the analysis of normal hepatocytes by flowcytometry is shown in Figure 1a. The majority of the cells are in the G0/G1 stage of the cell cycle and the DNA content of these cells represents the normal diploid compliment. However, on a quantitative basis, the average number of cells in the DEN-exposed animals with DNA content beyond diploid (Figure 1b.) was higher than in control animals (18,9% compared to 12.8%). Qualitatively, the descending side of the diploid G0/G1 population of cells from the DEN-exposed group was more diffuse and did not descent to baseline as clearly as in the control samples, and could represented the presence of inflammatory cells. The numbers of cells in the diploid G2 region also appear to be increased. This difference, however, will require additional studies for confirmation.

2. Detoxication Enzymes.

Two phases of detoxication are recognized (6) and enzymes representative of both phases were examined. Phase I metabolism, in which highly toxic compounds are converted to readily excreted polar compounds, is catalyzed by the mixed function oxidase (MFO) enzyme system. MFO-mediated reactions are considered important detoxication pathways, however, in a number of instances including key environmental contaminants, this pathway serves to activate substrates to more reactive, toxic products. Phase II enzymes catalyze the conjugation of substrates (usually polar intermediates generated by the Phase I pathway) with endogenous compounds such as glucose, amino acids or sulfate. The reactions comprising the Phase II metabolism serve to detoxify carcinogens via greatly enhanced excretability of the products.

EROD activity decreased significantly in the DEN-exposed group (Table 1). In contrast, GST activity increased over 40%, although the statistical significance of that increase was marginal. The general pattern of depressed Phase I activity and enhanced Phase II activity has been observed in fish following exposure to hepatotoxic agents (unpublished results).

3. Histopathology of DEN-Exposed Medaka.

Treatment-related changes were observed in the liver of male and female medaka that were repeatedly exposed to 200 mg/L DEN. The changes were characteristic of a toxic hepatopathy. In all treated fish there was moderate to moderately severe architectural disorganization characterized by loss of the usual tubular arrangement of hepatocytes and by variation in size of hepatocytes and hepatocytic nuclei. Other changes which were observed in variable numbers of treated fish include mild bile duct hyperplasia (male medaka), minimal to mild macrophage

aggregates and minimal to mild mononuclear cell foci. Cystic degeneration in the liver was moderate in three treated male medaka. One control male had minimal cystic degeneration. Hepatocellular vacuolation occurred in both control and treated medaka and the severity of vacuolation did not appear to be treatment related. Minimal hepatocyte hypertrophy occurred in one control female and moderate hepatocyte hypertrophy occurred in one treated male.

It is unclear, for example, if the effects we have described are related to a carcinogenic effects or merely reflect cellular toxicity associated with repeated exposure to high levels of DEN. The presence of proliferative biliary tissue suggests possible preneoplastic activity, although this is in no way definitive, as biliary hyperplasia is a common sequela to exposure to hepatotoxic agents. Cytotoxic effects have been shown to alter some of the molecular and biochemical responses measured in this study; for example, treatment of sunfish with hepatotoxic agents reduces contaminant-associated induction of EROD activity and results in increased levels of DNA strand breaks (unpublished results).

SUMMARY

The goal of this investigation was to describe genotoxic events at the molecular and biochemical level in the Japanese Medaka exposed to the well-characterized model carcinogens, diethylnitrosamine. Our studies focused on two key biochemical processes; DNA alterations and enzymes of the detoxification pathway, as well as effects observable histologically. As summarized in Table 1, DEN treatment inhibited Phase I enzyme activity and increased Phase II. DNA strand breaks were slightly elevated but no O^6-ethyl guanine adducts were detected. The presence of low levels of DNA strand breakage and no hypomethylation suggest an efficient DNA repair mechanism. Flow

cytometeric analyses show the DNA distribution in hepatocytes to be slightly abnormal (Figure 1). Histological evaluation indicated that repeated exposure of six month old Japanese medaka to 200 mg/L DEN resulted in toxic changes in the liver to include architectural disorganization in all treated fish, and bile duct hyperplasia, macrophage aggregates and mononuclear cell foci in variable numbers of treated fish. Further experiments affording longer grow-out time could provide a clearer correlation between the observed genotoxic events and neoplasia.

Acknowledgement:

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REFERENCES

1. Adamkiewics, J.; Eberle, G.; Huh, N.; Nehls, P.; Rajewsky, M. F. Quantitation and visualization of alkyl deoxynucleosides in the DNA of mammalian cells by monoclonal antibodies. Environ. Health Perspec. 62:49-55; 1985.
2. Burke, M. D.; Mayer, R. T. Ethoxyresorufin: Direct fluorimetric assay of a microsomal O-dealkylation which is preferentially induced by 3-methylcholanthrene. Drug Metab. Dispos. 2:583-588; 1974.
3. Cedar, H. DNA methylation and gene activity. Cell 53:3-4; 1988.
4. Habig, W. H.; Pabst, M. J.; Jacoby, W. B. Gluththione-S-Transferase: The first step in mercapturic acid formation. J. Biol. Chem. 249:7130-7139; 1974.
5. McBee, K.; Bickham, J. W. Petrochemical-related DNA damage in wild rodents detected by flow cytometry. Bull Environ. Contam. Toxicol. 40:343-349; 1987.
6. Payne, J. F. Mixed-function oxygenases in biological monitoring programs: Review of potential usage in different phyla of aquatic animals. In: Persoone, G.; Jaspers, E.; Claus, C., eds. Ecotoxicological testing for the marine environment. vol. 1. State Univ. Ghent and Inst. Mar. Scient. Res., Bredene, Belgium; 1984:625-655.
7. Shugart, L. R. Quantitation of chemically induced damage to DNA of aquatic organisms by alkaline unwinding assay. Aquat. Toxicol. 13:43-52; 1988.
8. Shugart, L. R. 5-methyl deoxycytidine content of DNA from bluegill sunfish exposed to benzo[a]pyrene. Environ. Toxicol. Chem. 9:205-208; 1990.
9. Shugart, L. R. Biological Monitoring: Testing for Genotoxicity. In: McCarthy, J. F.; Shugart, L. R. eds.

Biological Markers of Environmental Contaminants. Chelsea, MI: Lewis Pub. Inc., 1990-in press.

10. Shugart, L.R. DNA damage as an indicator of pollutant-induced genotoxicity. In: Landis, W. G.; van der Schalie, W. H., eds. 13th Symposium on Aquatic Toxicology and Risk Assessment: Sublethal Indicators of Toxic Stress. Philadelphia, PA: ASTM; 1990-in press.
11. Wogan, G. N.;Gorelick, N. J. chemical and biochemical dosimetry of exposure to genotoxic chemicals. Environ. Health Perspect. 62:5-18; 1985.

Table 1. Response of Molecular Markers in Japenese Medaka Exposed to Diethylnitrosame.

MOLECULAR MARKER	GROUP	
	Control	DEN-Exposed
DNA Alteration		
Strand breaks[a]	0.4 ± 0.04(10)	0.21 ± 0.12(30)
^{5}m-dCyd[b]	4.8	5.5
DNA Distribution	normal	tetraploidy
Detoxication		
Phase I-EROD[c]	8.2 ± 3.76(5)	2.70 ± 1.16(5)
Phase II-GST[d]	534 ± 88(5)	724 ± 213(6)

[a] Data reported as F values and are significantly different at alpha<0.001. Number of analyses in parenthesis.

[b] Data reported as % of total deoxycytidine content.

[c] Data reported as pmoles resorufin produced per min per mg of protein and are significantly different at alpha>0.05<0.001

[d] Data reported as units of enzyme activity per mg of protein and are significantly different at alpha>0.01<0.05

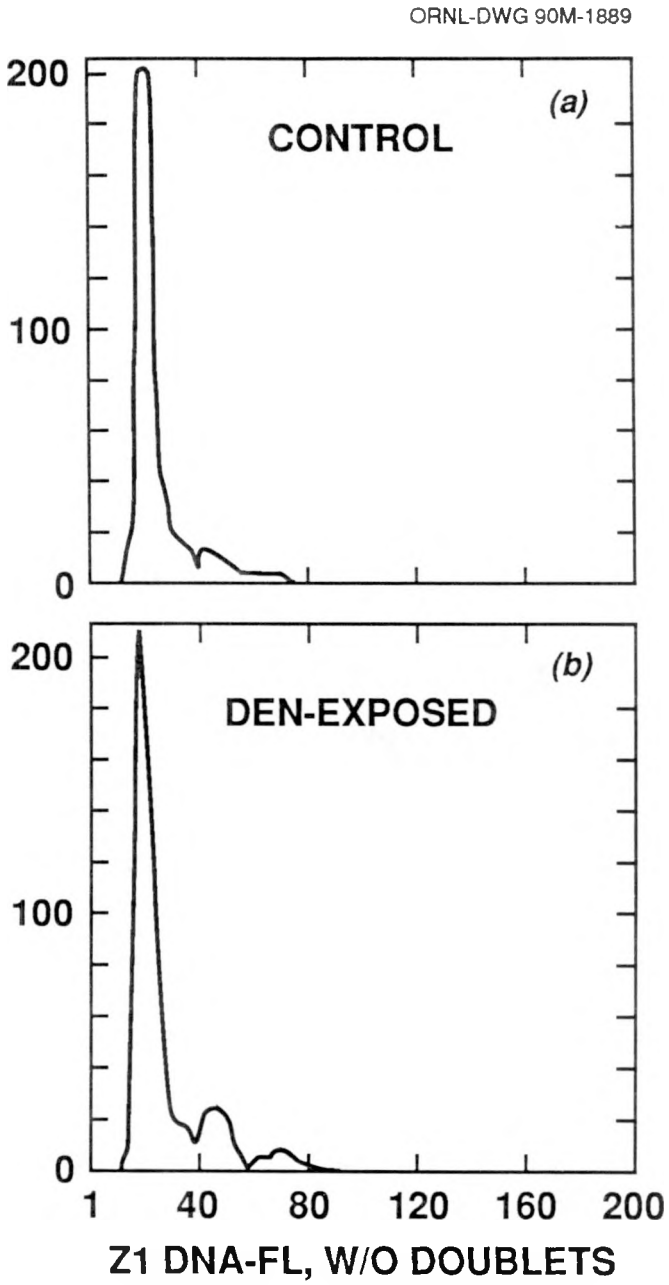

Fig.1 Frequency histograms of DNA content in hepatocytes. The results of analyses of Medaka hepatocytes from *(a)* control animals and *(b)* Den-exposed animals is shown. The major peak represents cells in the G1 (resting) stage of the cell cycle.

INTERNAL DISTRIBUTION

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17. USAMRDC, Attn: SGRD-ACQ, Fort Detrick, Frederick, Maryland 21701-5010

18. Office of Assistant Manager for Energy Research and Development, Oak Ridge Operations, P.O. Box 2001, U.S. Department of Energy, Oak Ridge, TN 37831-8600

19-28. Office of Scientific and Technical Information, P.O. Box 62, Oak Ridge, TN 37831-8600