REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

AD-A241 793

1. AG

3. REPORT TYPE AND DATES COVERED
FINAL 01 Nov 87 TO 31 Aug 91

4. TITLE AND SUBTITLE
MECHANISM OF LETHAL INTERACTION OF HAZARDOUS CHEMICALS AT SUBTOXIC DOSES

5. FUNDING NUMBERS
GR - AFOSR-88-0009
PE - 61102F
PR - 2313
TA - A5

(2)

6. AUTHOR(S)
Dr Harihara M. Mehendale

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Mississippi Medical Center
2500 North State Street
Jackson, Mississippi 39216-4505

8. PERFORMING ORGANIZATION REPORT NUMBER
AFOSR-TR- 91 0872

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Lt Col Cerveny
AFOSR/NL
Building 410
Bolling AFB DC 20332-6448

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited.

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 words) The possibility of unusual toxicity due to interaction of toxic chemicals upon environmental or occupational exposures to two or more chemicals, particularly when exposures involve levels ordinarily considered harmless individually is an important toxicological concern. Progress in this area of environmental toxicology has suffered for want of a model where the two interactants are individually nontoxic. Models, where toxic doses of chemicals are employed are not very representative of low level, environmental exposure. Prior exposure to nontoxic levels of the pesticide Kepone (chlordecone, CD) results in a 67-fold amplification of CCl4 lethality in experimental animals. This propensity for chlordecone to potentiate hepatotoxicity of halomethanes such as CCl4, CHCl3 and BrCCl3 has been the subject of this intense inquiry to unravel the underlying mechanism. the biological effects of this interaction include extensive hepatotoxicity characterized by histological alterations, hepatic dysfunction, and perturbation of related biochemical parameters. Mechanisms such as induction of microsomal cytochrome P-450 by chlordecone and greater lipid peroxidation are inadequate to explain the remarkably powerful potentiation of hepatotoxicity and lethality. Compelling experimental data from work completed thus far support the hypothesis that hepatocellular division during early time points after the administra-

14. SUBJECT TERMS

15. NUMBER OF PAGES

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
UNCLASSIFIED	UNCLASSIFIED	UNCLASSIFIED	UNLIMITED

tion of CCl4 is an important determinant of the progression (or regression) of the liver injury and consequent destruction (or restoration) of the hepatolobular architecture and function. A hypothesis for the mechanism of hepatotoxic and lethal effect of CCl4 as being primarily related to the accelerated progression of liver injury due to suppressed hepatocellular regeneration and hepatolobular restoration has been advanced in Medical Hypotheses, 33, 289-299, 1990. This is in contrast to the widely accepted putative mechanism, one which invokes only bioactivation followed by runaway lipid peroxidation as the events determining the course of the progressive phase of liver injury. The concept being advanced here accepts bioactivation and lipid perioxidation as the primary initiating events of cell injury. But proposes that the determinants of the progressive phase of liver injury are suppressed cell division and tissue repair. Incapacitation of the liver cells from regeneration is the determinant of the progression of liver injury, chich leads to the ultimate outcome of hepatotoxicity and lethality.

AFOSR-88-0009

MECHANISM OF LETHAL INTERACTION OF HAZARDOUS CHEMICALS AT SUBTOXIC DOSES

HARIHARA M. MEHENDALE, Ph.D.
DEPARTMENT OF PHARMACOLOGY AND TOXICOLOGY
UNIVERSITY OF MISSISSIPPI MEDICAL CENTER
2500 NORTH STATE STREET
JACKSON, MISSISSIPPI 39216-4505 USA

Telephone: 601-984-1618
Telefax: 601-984-1637

September 20, 1991

FINAL TECHNICAL REPORT
(Period covering November 15, 1987 - August 31, 1991 including 10 month extension without additional funding)

Distribution Statement

Prepared for:

T. Jan Cerveny, Ph.D., Lt. Col., USAF
Project Officer
Air Force Office of Scientific Research
Department of the Air Force
Bolling Air Force Base DC 20332-6448

A. Hypothesis: A two-stage model of toxicity

This research effort has resulted in the development of an hypothesis of a "two-stage model of toxicity". This concept has been expounded in an invited **"Commentary"** article which appeared recently in **Biochemical Pharmacology.**

Mehendale, H. M. **Commentary:** Role of hepatocellular regeneration and hepatocellular healing in the final outcome of liver injury. A two-stage model of toxicity. Biochem. Pharmacol. 42: 1155-1162, 1991.

In this article, the Principal Investigator has proposed that eventual outcome of toxicity is dependent on the events occurring in two stages. First stage (Stage I) consists of infliction of injury. The second stage consists of progression of injury (Stage II). Most of the mechanisms we presently understand as mechanisms of toxicity are really mechanisms of Stage I, meaning that these explain how toxicity is initiated. The mechanisms(s) of the progression of toxicity (suprathreshold) or regression of toxicity (infrathreshold) are determined by whether endogenous tissue repair is permitted to proceed allowing recovery from injury to occur, or are suppressed allowing a permissive progression of injury.

Development of this concept is likely to impact significantly on our view of toxicology and ultimately on how we go about the business of assessing risk to public health from exposure to toxic chemicals.

B. Executive Summary of Accomplishments

The possibility of unusual toxicity due to interaction of toxic chemicals upon environmental or occupational exposures to two or more chemicals, particularly when exposures involve levels ordinarily considered harmless individually is an important toxicological concern. Progress in this area of environmental toxicology has suffered for want of a model where the two interactants are individually nontoxic. Models, where toxic doses of chemicals are employed are not very representative of low level, environmental exposure. Prior exposure to nontoxic levels of the pesticide Kepone® (chlordecone, CD) results in a 67-fold amplification of CCl_4 lethality in experimental animals.

This propensity for chlordecone to potentiate hepatotoxicity of halomethanes such as CCl_4, $CHCl_3$ and $BrCCl_3$ has been the subject of this intense inquiry to unravel the underlying mechanism. The biological effects of this interaction include extensive hepatotoxicity characterized by histological alterations, hepatic dysfunction, and perturbation of related biochemical parameters. Mechanisms such as induction of microsomal cytochrome P-450 by chlordecone and greater lipid peroxidation are inadequate to explain the remarkably powerful potentiation of hepatotoxicity and lethality. Compelling experimental data from work completed thus far support the hypothesis that hepatocellular division during early time points after the administration of CCl_4 is an important determinant of the progression (or regression) of the liver injury and consequent destruction (or restoration) of the hepatolobular architecture and function. A hypothesis for the mechanism of hepatotoxic and lethal effect of CCl_4 as being primarily related to the accelerated progression of liver injury due to suppressed hepatocellular regeneration and hepatolobular restoration has been advanced in *Medical Hypotheses*, 33, 289-299, 1990. This is in contrast to the widely accepted putative mechanism, one which invokes only bioactivation followed by runaway lipid peroxidation as the events determining the course of the progressive phase of liver injury. The concept being advanced here accepts bioactivation and lipid perioxidation as the primary initiating events of cell injury, but proposes that the determinants of the progressive phase of liver injury are suppressed cell division and tissue repair. Incapacitation of the liver cells from regeneration is the determinant of the progression of liver injury, which leads to the ultimate outcome of hepatotoxicity and lethality.

C. Publications resulting from this project:

This technical report covers the grant period of 11/1/1987 - August 31, 1991.

This grant support has resulted in the following publications. These are listed as (a) original research publications: (i) published papers; (ii) in press papers; and (iii) submitted manuscripts. (b) Review articles and invited symposia presentations: (i) published; (ii) in press: and (c) Abstracts of paper presentations (i) published; (ii) in press and (iii) submitted abstracts.

a. Original research publications.

(i) Published papers.

1. Purushotham, K. R., Lockard, V. G. and Mehendale, H. M. Amplification of chloroform hepatotoxicity and lethality by dietary chlordecone (Kepone®) in mice. Toxicol. Pathol., 16: 27-34, 1988.

2. Mehendale, H. M., Purushotham, K. R. and Lockard, V. G. The time-course of liver injury and ^{3}H-thymidine incorporation in chlordecone-potentiated $CHCl_3$ hepatotoxicity. Exp. Mol. Pathol., 51: 31-47, 1989.

3. Rao, S. B. and Mehendale, H. M. Protective role of fructose 1,6-bisphosphate during CCl_4 hepatotoxicity in rats. Biochem. J., 262: 721-725, 1989.

4. Rao, S. B. and Mehendale, H. M. Protection from chlordecone-potentiated CCl_4 hepatotoxicity in rats by fructose 1,6-diphosphate. Int. J. Biochem., 21: 949-954, 1989.

5. Utley, W. S. and Mehendale, H. M. Phenobarbital-induced cytosolic cytoprotective mechanisms that offset increases in NADPH cytochrome P-450 reductase activity in menadione-mediated cytotoxicity. Toxicol. Appl. Pharmacol., 99: 323-333, 1989.

6. Rao, S. B., Young, R. A. and Mehendale, H. M. Polyamines and related enzymes following chlordecone-potentiated bromotrichloromethane hepatotoxicity in rats. J. Biochem. Toxicol., 5: 23-32, 1990.

7. Mehendale, H. M. and Ray, S. D. Inhibition of cell division in hepatoma cell cultures by chlordecone and carbon tetrachloride combination. Toxicol. *In Vitro*, 4: 179-184, 1990.

8. Ray, S. D. and Mehendale, H. M. Potentiation of CCl_4 and $CHCl_3$ hepatotoxicity and lethality by various alcohols. Fundam. Appl. Toxicol., 16: 429-440, 1990.

9. Utley, W. M. and Mehendale, H. M. Phenobarbital induced cytoprotective mechanisms in menadione metabolism: The role of glutathione reductase and DT-diaphorase. Int. J. Biochem., 22: 957-967, 1990.

10. Faroon, O. M. and Mehendale, H. M. Bromotrichloromethane hepatotoxicity. Role of hepatocellular regeneration in recovery. Biochemical and histopathological studies in control and chlordecone pretreated male rats. Toxicol. Pathol., 18: 667-677, 1990.

11. Kodavanti, P. R. S., Kodavanti, U. P. and Mehendale, H. M. CCl_4-induced alterations of hepatic calmodulin and free calcium levels in rats pretreated with chlordecone (Kepone®). Hepatology, 13: 230-238, 1991.

12. Abdul-Hussain, S. K. and Mehendale, H. M. Studies on the age-dependent effects of galactosamine on primary rat hepatocyte cultures. Toxicol. Appl. Pharmacol., 107: 504-513, 1991.

13. Utley, W. S. and Mehendale, H. M. Evidence for stimulated glutathione synthesis by phenobarbital pretreatment during an oxidative challenge in isolated hepatocytes. J. Biochem. Toxicol., 5: 101-113, 1991.

14. Faroon, O. M., Henry, R. W., Soni, M. G. and Mehendale, H. M. Potentiation of $BrCCl_3$ hepatotoxicity by chlordecone: biochemical and ultrastructural study. Toxicol. Appl. Pharmacol., 110: 185-197, 1991.

(ii) In Press Papers:

15. Soni, M. G. and Mehendale, H. M. Hepatic regeneration as a possible mechanism for the hepatoprotective action of cyanidanol. Int. J. Biochem., 23, In Press, 1991.

16. Mehendale, H. M., Ray, S. D., and Cai, Z. Paradoxical toxicity of CCl_4 in isolated hepatocytes from chlordecone, phenobarbital and mirex pretreated rats. *In vitro* Toxicol., 5: In Press, 1991.

17. Rao, V. C. and Mehendale, H. M. Colchicine antimitosis and its effect on CCl_4 autoprotection. Toxicol. Pathol. 20: In Press, 1991.

18. Rao, V. C. and Mehendale, H. M. Effect of colchicine on hepatobiliary function in CCl_4 treated rats. Biochem. Pharmacol. 43: In Press, 1992.

(iii) Submitted Manuscripts

19. Kodavanti, P. R. S., Kodavanti, U. P., Faroon, O. M. and Mehendale, H. M. Correlation of hepatocellular regeneration and CCl_4-induced hepatotoxicity in chlordecone, mirex or phenobarbital pretreated rats., Arch. Toxicol., Revised and Submitted, 1991.

20. Kodavanti, P. R. S., Rao, V. C., and Mehendale, H. M. Perturbations in calcium-regulated events that lead to progressive phase of chlordecone (Kepone®) potentiated CCl_4 hepatotoxicity. Hepatology, Submitted, 1991.

21. Soni, M. G. and Mehendale, H. M. Evidence for hepatic failure in the interactive toxicity of chlordecone and carbon tetrachloride. Toxicol. Appl. Pharmacol., Submitted, 1991.

22. Abdul-Hussain, S. K. and Mehendale, H. M. Biochemical studies on the age-related toxicity of galactosamine in primary rat hepatocyte cultures. Toxicol. *In Vitro*, Submitted, 1991.

23. Soni, M. G. and Mehendale, H. M. Adenosine triphosphate protections of chlordecone-amplified CCl_4 hepatotoxicity and lethality. J. Hepatol., Submitted, 1991.

b. Review Articles and Invited Symposia Presentations:

(i) Published articles.

24. Mehendale, H. M. Amplification of hepatotoxicity and lethality of CCl_4 and $CHCl_3$ by chlordecone. Rev. Biochem. Toxicol., 10: 91-138, 1989.

25. Mehendale, H. M. Impact of chemical interactions on the development of cancer. ACS Symposium Series. 414, *Carcinogenicity of Pesticides,* Chapter 8, 122-141, 1989.

26. Utley, W. S. and Mehendale, H. M. Cytoprotective mechanisms that offset phenobarbital-induced increment in $O^{\cdot}_2$ generated from quinone recycling in *Proceedings of the International Conference in "Biological Oxidation Systems"* (Ed. C. C. Reddy, G. Hamilton and K. M. Madyastha) Academic Press, New York, NY. Vol. I: 183-200, 1990.

27. Mehendale, H. M. Potentiation of halomethane hepatotoxicity by chlordecone: A hypothesis for the mechanism. Med. Hypoth. 33: 289-299, 1990.

28. Mehendale, H. M. Hepatocellular regeneration as a determinant of halomethane toxicity. In *Proceedings of the International Conference In "Biological Oxidation Systems"* (Ed. C. C. Reddy, G. Hamilton and K. M. Madyastha) Academic Press, New York, NY. Vol. II: Chapter VIII, 1017-1036, 1990.

29. Kodavanti, P. R. S. and Mehendale, H. M. Biochemical methods of studying hepatotoxicity. In *Hepatotoxicology* (Ed. R. G. Meeks, S. D. Harrison, and R. J. Bull) CRC Press, Times Mirror Publishing Company, Boca Raton, FL, Chapter 7, 241-325, 1991.

30. Mehendale, H. M. Commentary: Role of hepatocellular regeneration and hepatolobular healing in the final outcome of liver injury: A two-stage model of toxicity. Biochem. Pharmacol., 42: 1155-1162, 1991.

(ii) In Press Papers

31. Soni, M. G. and Mehendale, H. M. Role of perturbed hepatocellular energy status in chlordecone-amplification of CCl_4 toxicity. Ind. J. Environ. Toxicol., 1: In Press, 1991.

32. Mehendale, H. M. Biochemical mechanisms of biphasic dose-response relationships: Role of hormesis. In: *Toxicological Implications of Biological Adaptations* (Ed. E. J. Calabrese), Lewis Publishers, chelsea, MI, In Press, 1992.

(iii) Submitted manuscript

33. Mehendale, H. M. Toxicology of chemical combinations: A mechanistic perspective. Everyman's Science, Submitted, 1991.

c. Abstracts of paper presentations

(i) Published

1). Prasada Rao, K. S., Joshi, U. M., and Mehendale H. M. Hepatic energy status during CCl_4 toxicity in rats pretreated with chlordecone, mirex and phenobarbital. Toxicologist, 8: 66, 1988.

2). Menendale, H. M. and Rao, S. B. Protective role of fructose 1,6-diphosphate during CCl_4 hepatotoxicity Am. Chem. Soc. Abstr.. #167, 1988.

3). Prasada Rao, K. S. and Mehendale, H. M. Correlation of hepatocellular regeneration and CCl_4 hepatotoxicity in chlordecone, mirex or phenobarbital pretreated rats. FASEB J. 2: A408, 1988.

4). Kodavanti, P. R. S. and Mehendale, H. M. Role of hepatic c-AMP and phosphorylase levels in chlordecone (Kepone®)-potentiated CCl_4 hepatotoxicity. FASEB J. 2: A1037, 1989.

5). Kodavanti, P. R. S., Joshi, U. M. and Mehendale, H. M. CCl_4-induced alterations of hepatic calmodulin in rats pretreated with chlordecone (Kepone®). Vth IUTOX Meetings, Brighton, England, July 16-21, 1989.

6). Mehendale, H. M. and Ray, S. D. Suppression of cell division in Reuber hepatoma cells pre-exposed to chlordecone by CCl_4. Toxicologist, 9: 68, 1989.

7). Mehendale, H. M. and Ray, S. D. Chlordecone pretreatment but not mirex or phenobarbital inhibit Reuber hepatoma cell division upon exposure to CCl_4. Vth IUTOX Meetings, Brighton, England, July 16-21, 1989.

8). Ray, S. D. and Mehendale, H. M. Influence of phenobarbital, mirex and chlordecone on the effect of CCl_4 on Reuber hepatoma cell growth. FASEB J. 2: A1037, 1989.

9). Ray, S. D. and Mehendale, H. M. Potentiation of carbon tetrachloride hepatotoxicity and lethality by various alcohols. Toxicologist, 9: 59, 1989.

10). Utley, W. S. and Mehendale, H. M. The contribution of DT-diaphorase in hepatocytes isolated from naive and phenobarbital pretreated rats during menadione metabolism. FASEB J. 2: A919, 1989.

11). Utley, W. S. and Mehendale, H. M. Evidence for enhanced DT-diaphorase-mediated metabolism of menadione by phenobarbital pretreatment. Toxicologist, 9: 26, 1989.

12). Faroon, O. M. and Mehendale, H. M. Ultra structure and biochemical studies of chlordecone-potentiated bromotrichloromethane ($BrCCl_3$) hepatotoxicity. Toxicologist, 10: 61, 1990.

13). Ray, S. D., Cai, Z., and Mehendale, H. M. Paradoxical toxicity of CCl_4 in isolated hepatocytes from chlordecone, phenobarbital and mirex pretreated rats. Toxicologist, 10: 53, 1990.

14). Utley, W. S. and Mehendale, H. M. Phenobarbital-mediated increases in GSH synthesis in isolated hepatocytes incubated with menadione. Toxicologist, 10: 23, 1990.

15. Abdul-Hussain, K. S. and Mehendale, H. M., Biochemical studies on the age-related toxicity of galactosamine in primary hepatocyte culture. Pharmacologist, 32: 16, 1990.

16. Rao, V. C. and Mehendale, H. M. Effect of antimitotic agent colchicine on CCl_4 toxicity. Pharmacologist, 32: 168, 1990.

17. Faroon, O. M., Henry, R. W., Soni, M. G., and Mehendale, H. M. Potentiation of bromotrichloromethane hepatotoxicity in male rats exposed to 10 ppm dietary chlordecone: Electron microscopic and biochemical study. Ann. Proc. Elect. Microsc. Soc. Amer., 3: 284, 1990.

18. Cai, Z. and Mehendale, H. M. Prestimulation of hepatocellular regeneration by partial hepatectomy decreases CCl_4 toxicity in gerbils. Toxicologist, 11: 65, 1991.

19. Rao, V. C. and Mehendale, H. M. Prolongation of carbon tetrachloride toxicity by colchicine antimitosis. Toxicologist, 11: 128, 1991.

20. Kodavanti, U. P., Sharma, R. and Mehendale, H. M. In vivo inhibition of pulmonary and macrophage phospholipase in drug-induced phospholipidosis. Toxicologist, 11: 153, 1991.

21. Abdul-Hussain, S. K. and Mehendale, H. M. Ongoing hepatocellular regeneration and resiliency towards galactosamine hepatotoxicity. Toxicologist, 11: 169, 1991.

22. Soni, M. G. and Mehendale, H. M. Evidence for hepatic failure during chlordecone-amplified CCl_4 toxicity. Toxicologist, 11: 169, 1991.

23. Thakore, K. N. and Mehendale, H. M. Effect of phenobarbital (PB) and mirex (M) on CCl_4-autoprotection. Toxicologist, 11: 169, 1991.

24. Mehendale, H. M. and Cai, Z. Resiliency of developing rats to potentiation of hepatotoxicity and lethality of CCl_4 by chlordecone. Toxicologist, 11: 219, 1991.

25. Soni, M. G. and Mehendale, H. M. Adenosine triphosphate protection of chlordecone amplified CCl_4 hepatotoxicity and lethality. FASEB J., 5: A1604, 1991.

26. Thakore, K. N. and Mehendale, H. M. Liver injury and CCl_4 autoprotection. FASEB J., 5: A1248, 1991.

27. Aroor, A. R. and Mehendale, H. M. The role of polyadenosine diphosphate ribose polymerase (PARP) activity in carbon tetrachloride hepatotoxicity. FASEB J., 5: A1615, 1991.

28. Rao, C. V., Kodavanti, P. R. S., Kodavanti, U. P. and Mehendale, H. M. Perturbation of calcium regulated events during chlordecone (CD) potentiated CCl_4 hepatotoxicity. FASEB J., 5: A1615, 1991.

29. Cai, Z. and Mehendale, H. M. Role of on going versus stimulated hepatocellular regeneration in resiliency to amplification of CCl_4 toxicity by chlordecone. FASEB J., 5: A1248, 1991.

(ii) In Press Abstract

30. Soni, M. G. and Mehendale, H. M. Protection against the interactive hepatotoxicity and lethality of chlordecone and carbon tetrachloride by administration of ATP. Hepatology, 14: In Press, 1991.

(iii) Submitted, by Invitation

31. Mehendale, H. M. Amplified interactive toxicity of chemicals at nontoxic levels: Mechanistic considerations and implications to public health. For symposia presentation at a satellite meeting of IUTOX-VI ICT, entitled, "Toxicological Evaluation of Chemical Interactions: Relevance of social, environmental and occupational factors", organized by IV European ISSX Meeting at Bologna, Italy, July 4-6, 1992.

D. Recent Preliminary Studies.

In order to investigate the mechanism(s) responsible for the failure of the hepatocytes to extrude extracellular Ca^{2+} in the CD + CCl_4 interaction, we are proposing to study the Ca^{2+}-ATPase pumps in isolated hepatocellular plasma membrane preparations. The plasma membranes will be used to measure Ca^{2+}-ATPase activities. Hepatocytes isolated from variously treated rats at various times after the administration of halomethanes will be used to obtain plasma membranes, which will be assayed for Ca^{2+}-ATPase activity. Preliminary work establishing this technique was included in the competitive renewal application submitted in April, 1991.

Preliminary studies were carried out to find out the reliability and efficiency of estimation of Ca^{2+}-ATPase from plasma membranes isolated from hepatocytes. The preparations was found to be over 95% pure and the results were repeatable. A brief report on standardization of estimation of Ca^{2+}-ATPase follows.

Efficiency of estimation of Ca^{2+} ATPase activity:

It was observed that, greater the viability of cells, more will be the attachment to the beads. In order to find out the reliability of the experiment, the beads were incubated with hepatocytes with greater than 85% viability and with hepatocytes killed by adding 8 mM CCl_4. It was observed that the hepatocytes

with greater than 85% viability showed very high efficiency of attachment and the cells killed by CCl_4 showed very poor degree of attachment. The findings show that if the viability of the cells is decreased to begin with, the attachment to beads also decreases. These experiments indicate that the dead cells do not attach to the beads. Therefore, it can be said that the activity of Ca^{2+}-ATPase in isolated hepatocyte plasma membrane preparations is from live cell membranes rather than from the dead cells.

Furthermore, purity of the membrane preparation and reliability of membrane isolation technique tested by carrying out experiments on marker enzyme activities, such as 5^1-nucleotidase as a marker for the plasma membrane, succinate dehydrogenase for mitochondrial, and glucose-6-phosphatase for microsomal source. These enzyme activities were estimated in the membrane preparations at different stages of isolation. After establishing that the isolation technique employed for obtaining pure plasma membrane, Ca^{2+}-ATPase activity was measured in the membranes isolated from hepatocytes. Studies were carried out by treating rats with CCl_4, with or without prior dietary exposure to chlordecone (Kepone^R). These studies are described below.

Animal Treatment:

Harlan Sprague-Dawley, male rats weighing 200-250g maintained on normal diet (purina chow) were given intraperitoneal injection of 100 μl CCl_4/kg in corn oil and the hepatocytes were isolated for estimation of Ca^{2+}-ATPase activity in plasma membrane as explained under Methods section. Enzyme activity was estimated at different time points after injection, such as 0, 1, 2, 4, 6 and 24 hrs. Similarly, controls were given plain corn oil (0.1 ml/kg) and the Ca^{2+}-ATPase activity was measured in the isolated plasma membranes at different time points.

Another group of rats weighing 175-200 g were maintained on 10 ppm chlordecone (CD) diet for 15 days and were intraperitoneally injected with 100 μl CCl_4/kg in corn oil after 15 days and the Ca^{2+} ATPase activity in plasma membrane of hepatocytes was estimated at different time points of 0, 1, 2, 4, 6 and 24 hrs. Similarly, controls such as only CD treated animals were given (0.1 ml/kg) corn oil and the ts Ca^{2+}-ATPase activity was measured. Only the results of the study carried out after CCl_4 and CD + CCl_4 treatment at 2 and 4 hr time points were reported in the competitive application.

From these studies, it is evident that CD and CCl_4 interaction causes alteration in membrane Ca^{2+} ATPase activity. Scope of this study was to establish that Ca^{2+}-ATPase activity could be measured in purified plasma membranes under moderate or severe toxicity. CCl_4 alone at 100 μl/kg is not known to cause much toxicity. Same dose given to CD treated rats is known to be severely toxic and intracellular Ca^{2+} is known to rise dramatically.

E. Summary of the Proposed Work in the Pending Competitive Renewal Proposal

The primary objective of the competitive renewal proposal is to investigate the mechanism of CD-amplified halomethane (CCl_4, $CHCl_3$, $BrCCl_3$, etc) hepatotoxicity. The mechanism(s) leading to the failure of cell division and tissue repair is (are) the investigational target of this proposal. The working hypothesis is that CD sensitizes the liver tissue to greatly perturb plasma membrane Ca^{2+} extrusion mechanism leading to greatly amplified disruption of Ca^{2+} homeostasis in the liver cells. The consequence is a cascade of biochemical events, leading to a dramatic depletion of cellular energy, along with Ca^{2+}-mediated activation of biochemical disruption. Two main eventualities result from this. First, hepatocellular death occurs leading to initially a centrilobular necrosis. Second, there is a decisive biochemical disruption in the other living cells. Ultimately, these events incapacitate the cells from responding to injury by way of cell division and tissue repair.

Molecular mechanisms responsible for arrested cell division and tissue repair are also of investigational interest regardless of the involvement of Ca^{2+}. To this end, we have established an *in vitro*

model with hepatoma cell line, which will be further developed to explore and pin-point the disrupted step or steps in cell-cycling, and related molecular biology. We will measure intracellular Ca^{2+} distribution, hepatocellular cytosolic free Ca^{2+}, and determine the role of mitochondria and smooth endoplasmic reticulum in the disruption of calcium homeostasis. Mirex (M) and phenobarbital (PB) pretreatments will be used as negative and positive controls, respectively. These concepts will also be subjected to experimental validation by additional histomorphometric studies of M + CCl_4 and PB + CCl_4 treatments and also with studies of $BrCCl_3$ potentiation. The long-term objective is to unravel the mechanism of this unusually powerful toxic interaction between CD + CCl_4 at individually subtoxic doses. Furthermore, since CD is also known to potentiate the toxicity of $CHCl_3$ and $BrCCl_3$, the halomethanes related the CCl_4, this proposal is intended to test our hypothesis on the mechanism of highly amplified toxicity of the halomethanes by CD in order that the mechanistic concepts are subjected to experimental verification.