CHLORAL HYDRATE CARDIOTOXICITY IN ADULT AND NEONATAL RABBIT HEARTS

A Thesis Submitted to the College of
Graduate Studies and Research
in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy
in the Division of Pharmaceutical Sciences
College of Pharmacy and Nutrition
University of Saskatchewan
Saskatoon, Canada

By

Sheri Lynne Fandrey, B.S.P.

Spring 1997

UNIVERSITY OF SASKATCHEWAN

College of Graduate Studies and Research

SUMMARY OF DISSERTATION

Submitted in partial fulfillment
of the requirements for the

DEGREE OF DOCTOR OF PHILOSOPHY

by

Sheri L. Fandrey

Division of Pharmaceutical Sciences
College of Pharmacy and Nutrition
University of Saskatchewan

Fall 1996

Examining Committee:

Dr. R. Khandelwal	Dean's Designate, Chair College of Graduate Studies and Research
Dr. E.M. Hawes	Chair of Advisory Committee, College of Pharmacy and Nutrition
Dr. D.K.J. Gorecki	Co-supervisor, College of Pharmacy and Nutrition
Dr. K.W. Hindmarsh	Co-supervisor, Faculty of Pharmacy, University of Manitoba, Winnipeg, Manitoba
Dr. K. Prasad	Department of Physiology, College of Medicine
Dr. S.M. Wallace	College of Pharmacy and Nutrition
Dr. H.A. Semple	College of Pharmacy and Nutrition
Dr. J. Hubbard	College of Pharmacy and Nutrition

External Examiner:

Dr. T. Massey
Department of Pharmacology and Toxicology
Queen's University
Kingston, Ontario
K7L 3N6

CHLORAL HYDRATE CARDIOTOXICITY IN ADULT AND NEONATAL RABBIT HEARTS

Chloral hydrate (CH) is one of the most commonly used sedative-hypnotic agents in pediatric patients. Neonatal patients tend to accumulate CH metabolites trichloroethanol (TCE) and trichloroacetic acid (TCA), leading to the concern that CH administration may contribute to the development of cardiac arrhythmias in this population. The present investigation has focused, using an *in vitro* rabbit heart model, on the mechanism and developmental aspects of cardiotoxicity of clinically relevant concentrations of CH, TCE and TCA.

The results show that CH, TCE and TCA are general cardiac depressants in the isolated perfused adult rabbit heart. CH and its metabolites also caused atrio-ventricular and intra-ventricular conduction delays. This type of phenomenon is involved in the development of re-entrant cardiac arrhythmias. A number of arrhythmias, including atrial and ventricular fibrillation, were observed in the hearts treated with TCE and TCA. The cardiac depressant effects were much less pronounced in the isolated perfused neonatal rabbit heart. CH, TCE and TCA produced essentially no conduction delays or serious arrhythmias in the neonatal heart.

CH and TCA produced increases in thiobarbituric acid reactive substances (TBARS) in adult cardiac tissue, suggesting that lipid peroxidation is initiated by these compounds. TBARS levels were also increased following TCE and TCA treatment in juvenile cardiac tissue. Neonatal myocardium appeared to be protected from the peroxidative effects of CH and its metabolites, with TBARS levels significantly reduced in the treated tissue. The reduced lipid peroxidation in neonatal hearts may be related to significantly increased activities of three antioxidant enzymes in the neonatal myocardium.

It was demonstrated that the heart is a site of metabolism and accumulation of chloral hydrate and its two primary metabolites in both neonatal and adult hearts. The heart therefore appears to be capable of producing reactive metabolites of chloral hydrate which may be involved in lipid peroxidation and subsequent cardiotoxic effects.

In conclusion, the present investigation has shown that the cardiotoxic effects of chloral hydrate and its metabolites may be related to myocardial lipid peroxidation. Myocardial accumulation and metabolism of CH, TCE and TCA may contribute to the cardiotoxic effects of these compounds. The resistance of the neonatal heart to cardiotoxic effects of CH and its metabolites may be due to high levels of antioxidant enzymes in immature *versus* adult hearts.

BIOGRAPHICAL

Education

April, 1990	B.S.P. (Great Distinction), University of Saskatchewan

Honours

1995	Hoechst-Roussel Canada Incorporated Graduate Award
	Alf Pepper Research Award
1994	University of Saskatchewan Graduate Scholarship
1993	Parke-Davis Centennial Pharmacy Research Award
1992	PMAC - HRF Scholarship (2 year award)
	Heart & Stroke Foundation Scholarship (declined)
1991	Parke-Davis Centennial Pharmacy Research Award
1990	F.J. Fear Scholarship
	PMAC - HRF Scholarship (2 year award)
1989	Dean's Honor Roll
1987-1989	MRC Farquharson Research Scholarships (3)
1986	Dean W.C. MacAulay Memorial Bursary
1985	University Undergraduate Scholarship
1983	Western Machine & Engineering, Limited Book Prize
1982	University Entrance Scholarship
	Governor General's Bronze Medal

PUBLICATIONS

Research Articles:

(1) KW Hindmarsh, A Taylor, SL Fandrey. Solvent abuse - changes in attitudes and knowledge? Submitted to Canadian Society of Forensic Sciences Journal, June 1996.

(2) SL Fandrey, K Prasad, HA Semple, KW Hindmarsh, DKJ Gorecki. Effects of chloral hydrate and its metabolites in the isolated, perfused adult rabbit heart. Manuscript in preparation for submission to Journal of Pharmacology and Experimental Therapeutics, 1996.

(3) SL Fandrey, K Prasad, HA Semple, KW Hindmarsh, DKJ Gorecki. Effects of chloral hydrate and its metabolites in the isolated, perfused neonatal rabbit heart. Manuscript in preparation for submission to Journal of Pharmacology and Experimental Therapeutics, 1996.

(4) SL Fandrey, HA Semple, KW Hindmarsh, DKJ Gorecki. A comparative study of the disposition and accumulation of chloral hydrate and its metabolites in adult and neonatal rabbit hearts. Manuscript in preparation for submission to Drug Metabolism and Distribution, 1996.

(5) SL Fandrey, SV Mantha, K Prasad, KW Hindmarsh, DKJ Gorecki. Developmental aspects of chloral hydrate cardiotoxicity: lipid peroxidation and anti-oxidant status. Manuscript in preparation for submission to Free Radical Biology and Medicine, 1996.

(6) JR Dimmock, JM McColl, SL Wonko (Fandrey), RS Thayer, and DS Hancock. Evaluation of the thiosemicarbazones of some aryl alkyl ketones and related compounds for anticonvulsant activities, European Journal of Medicinal Chemistry **26**, 528-534, 1991.

(7) JR Dimmock, VK Arora, SL Wonko (Fandrey), NW Hamon, JW Quail, Z Jia, RC Warrington, WD Fang, and JS Lee. 3,5-*bis* -Benzylidene-4-piperidones and related compounds with high activity towards P388 leukemia cells, Drug Design and Delivery **6**, 183-194, 1990.

(8) JR Dimmock, OA Phillips, SL Wonko (Fandrey), RA Hickie, RG Tuer, SJ Ambrose, RS Reid, B Mutus, and CJ Talpas. Evaluation of some Mannich bases of conjugated styryl ketones and related compounds *vs.* the WiDr colon cancer *in vitro*, European Journal of Medicinal Chemistry **24**, 217-226, 1989.

Abstracts:

(1) SL Fandrey, SV Mantha, K Prasad, DKJ Gorecki, KW Hindmarsh. Developmental aspects of chloral hydrate cardiotoxicity: lipid peroxidation and anti-oxidant status, Pharmaceutical Research, accepted for publication July 1996.

(2) SL Fandrey, DKJ Gorecki, KW Hindmarsh, K Prasad. Effects of chloral hydrate and its metabolites on the isolated, perfused, neonatal rabbit heart, Pharmaceutical Research, **12**(9):S403, 1995.

(3) SL Fandrey, DKJ Gorecki, HA Semple, K Prasad. Disposition and accumulation of chloral hydrate and its metabolites in the isolated perfused rabbit heart, Pharmaceutical Research, **10**(10):S369, 1993.

(4) SL Fandrey, KW Hindmarsh, DKJ Gorecki, K Prasad. Effects of chloral hydrate and its metabolites on the isolated, perfused rabbit heart, Pharmaceutical Research, **9**(10):S277, 1992.

PERMISSION TO USE

In presenting this thesis in partial fulfillment of the requirements for a Postgraduate degree from the University of Saskatchewan, I agree that the Libraries of this University may make it freely available for inspection. I further agree that permission for copying of this thesis in any manner, in whole or in part, for scholarly purposes may be granted by the professor or professors who supervised my thesis work or, in their absence, by the Head of the Department or the Dean of the College in which my thesis work was done. It is understood that any copying or publication or use of this thesis or parts thereof for financial gain shall not be allowed without my written permission. It is also understood that due recognition shall be given to me and to the University of Saskatchewan in any scholarly use which may be made of any material in my thesis.

Requests for permission to copy or to make other use of material in this thesis in whole or part should be addressed to:

Dean of the College of Pharmacy and Nutrition
University of Saskatchewan
110 Science Place
Saskatoon, Saskatchewan
Canada
S7N 5C9

ABSTRACT

Chloral hydrate (CH) is one of the most commonly used sedative-hypnotic agents in pediatric patients. Previous investigations led to the concern that accumulation of chloral hydrate metabolites trichloroethanol (TCE) or trichloroacetic acid (TCA) in neonatal patients may have toxic potential. Of particular concern is the possibility that CH, TCE and/or TCA may contribute to the development of cardiac arrhythmias in this population. The present investigation has focused, using an *in vitro* rabbit heart model, on the mechanism and developmental aspects of cardiotoxicity of clinically relevant concentrations of CH, TCE and TCA.

The results show that CH, TCE and TCA are general cardiac depressants in the isolated perfused adult rabbit heart. In the adult rabbit heart, all three compounds depressed coronary flow, contractility, and myocardial oxygen consumption over the range of concentrations tested. CH and its metabolites also caused conduction defects which are consistent with atrio-ventricular and intra-ventricular conduction delays. This type of phenomenon is involved in the development of re-entrant cardiac arrhythmias. A number of arrhythmias, including atrial and ventricular fibrillation, were observed in the hearts treated with TCE and TCA.

The cardiac depressant effects were much less pronounced in the isolated perfused neonatal rabbit heart. CH, TCE and TCA had minimal and unpredictable effects on left ventricular contractility and oxygen consumption. Of particular importance was the observation that CH, TCE and TCA produced essentially no conduction delays in the neonatal heart. Few serious arrhythmias could be attributed to factors other than incidental conduction delays in the neonatal rabbit heart.

Biochemical and pharmacological data suggest the involvement of free radicals in lipid peroxidation of phospholipid components of the cell membrane in the adult heart preparations. CH and TCA produced increases in thiobarbituric acid reactive substances (TBARS) in adult cardiac tissue,

suggesting that lipid peroxidation is initiated by these compounds. TBARS levels were also increased following TCE and TCA treatment in juvenile cardiac tissue. Neonatal myocardium appeared to be protected from the peroxidative effects of CH and its metabolites, with TBARS levels significantly reduced in the treated tissue. The reduced lipid peroxidation in neonatal hearts may be related to significantly increased activities of three antioxidant enzymes - catalase, glutathione peroxidase and superoxide dismutase - in the neonatal myocardium.

It was demonstrated that the heart is a site of metabolism and accumulation of chloral hydrate and its two primary metabolites in both neonatal and adult hearts. It is axiomatic that the effects of chemically reactive intermediates tend to be limited to the tissues in which they are formed. Also, very low concentrations of reactive intermediates have demonstrated significant toxicity *in vivo*. Therefore, the heart appears to be capable of producing reactive metabolites of chloral hydrate which may be involved in lipid peroxidation and subsequent cardiotoxic effects.

In conclusion, the present investigation has shown that the cardiotoxic effects of chloral hydrate and its metabolites may be related to myocardial lipid peroxidation. Myocardial accumulation and metabolism of CH, TCE and TCA may contribute to the cardiotoxic effects of these compounds. The resistance of the neonatal heart to cardiotoxic effects of CH and its metabolites may be due to high levels of antioxidant enzymes in immature *versus* adult hearts.

ACKNOWLEDGEMENTS

I would like to acknowledge the following people for their contributions to the successful completion of my thesis project:

Drs. D.K.J. Gorecki and K.W. Hindmarsh, without whose supervision, guidance, encouragement and humour this project would not have occurred. I could not have selected better co-supervisors. Thank you both!

Dr. E.M. Hawes, as the chair of my Advisory Committee, your tireless assistance in all things administrative and bureaucratic made my program proceed as smoothly as possible.

Dr. S.M. Wallace, from statistics and pharmacokinetics to the Brontë sisters and Graham Kerr, you have my admiration in all things.

Dr. H.A. Semple, your substantial contributions to the development and completion of my project, as well as my development as a scientist, cannot be over-estimated.

Dr. K. Prasad, my "other" supervisor - your generosity of both time and resources were absolutely invaluable.

Dr. J. Hubbard, your thoughtful critique of my thesis under serious time constraints was very much appreciated.

Dr. S.V. Mantha, your expert technical assistance and suggestions added a depth and significance to my project that would otherwise have been lacking.

Ray Risling and Peggy Wallace, your capable assistance in all aspects of rabbit handling, surgery and husbandry was greatly appreciated.

Barbara Raney, your friendship and encouragement mean more than you know - thanks!

Dr. Elaine Blau, your support, assistance and friendship are unconditional. You add to my life in so many ways. Thank you for everything.

My friends and colleagues in the College of Pharmacy and Nutrition and elsewhere are simply too numerous to mention. Your time, understanding and support made my life immeasurably brighter. Thank you all!

And for Chica - who has been there through it all!

DEDICATION

I dedicate this thesis to
my parents Clare and Lois
and
my husband Blair.
There are simply not enough words.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

2° AV block - Type I or II	Type I or II second-degree atrio-ventricular block
3° AV block	Third-degree atrio-ventricular block
ANOVA	Analysis of variance
A_{SS}	Amount of drug accumulated at steady-state
ATP	Adenosine triphosphate
ATPase	Adenosine triphosphatase
AUC	Area under the curve
AUMC	Area under the first moment curve
BBB	Bundle branch block
Ca^{2+}	Calcium ion
CAT	Catalase
CCAC	Canadian Council on Animal Care
CF	Coronary flow
CH	Chloral hydrate
CL	Clearance
C_{max}	Maximal concentration of drug
cmH_2O	Centimeters of water (a measure of pressure)
CNS	Central nervous system
CO_2	Carbon dioxide
C_{SS}	Concentration of drug at steady-state
Cu/ZnSOD	Copper/zinc superoxide dismutase
CYP2E1	Cytochrome P450IIE1 isozyme
DCA	Dichloroacetic acid
DNA	Deoxyribonucleic acid
dP/dT	First derivative of LVDP
Dur'n	Duration
e^-	Electron
EC	Enzyme Commission International

ECD	Electron-capture detector
ECG	Electrocardiograph
FeSOD	Iron superoxide dismutase
GRAS	Generally regarded as safe
GSH	Reduced glutathione
GSH-Px	Glutathione peroxidase
GSSG	Oxidized glutathione
H^+	Hydrogen ion
H_2O	Water
H_2O_2	Hydrogen peroxide
$HO_2^{\bullet}$	Hydroperoxyl radical
HR	Heart rate
JEB	Junctional escape beat
k_0	Input rate
LVDP	Left ventricular developed pressure
LVEDP	Left ventricular end diastolic pressure
LVSP	Left ventricular systolic pressure
mmHg	millimeters of mercury (a measure of pressure)
MnSOD	Manganese superoxide dismutase
MPTP	1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MRT	Mean residence time
MTCA	Methyltrichloroacetate
MVO_2	Myocardial oxygen consumption
NAD(H)	Nicotinamide adenine dinucleotide
NADP(H)	Nicotinamide adenine dinucleotide phosphate
O_2	Molecular oxygen
$O_2^{\bullet-}$	Superoxide anion
OH^-	Hydroxyl anion
$^{\bullet}OH$	Hydroxyl radical
P450	Cytochrome P450

PAC	Premature atrial contraction
PD	Pharmacodynamic(s)
PK	Pharmacokinetic(s)
PNS	Parasympathetic nervous system
PSVT	Paroxysmal supraventricular tachycardia
PUFA	Polyunsaturated fatty acid
PVC	Premature ventricular contraction
Rec.	Recovery (phase)
RNA	Ribonucleic acid
ROS	Reactive oxygen species
S.E. (S.E.M.)	Standard error (of the mean)
SNS	Sympathetic nervous system
SOD	Superoxide dismutase
ST	Sinus tachycardia
SVT	Supraventricular tachycardia
T	Time
TBA	Thiobarbituric acid
TBARS	Thiobarbituric acid-reactive substance
TCA	Trichloroacetic acid
TCE	Trichloroethanol
TCP	1,2,3-Trichloropropane
T_{max}	Time to reach maximal drug concentration
Tx	Treatment (phase)
V. bigeminy/VB	Ventricular bigeminy
V. fibrillation/VF	Ventricular fibrillation
V. tachycardia/VT	Ventricular tachycardia
VEB	Ventricular ectopic beat
V_{ss}	Apparent volume of distribution at steady-state

1. INTRODUCTION

Human fetuses and neonates are constantly exposed to xenobiotics, either inadvertently via maternal ingestion or exposure, or directly as therapeutic measures. However, until approximately thirty years ago human infants were considered pharmacologically to be merely miniature adults. That this overly simplistic view is therapeutically untrue was unfortunately illustrated by several tragedies in children resulting from drug administration. Therapeutic misadventures such as the "grey baby syndrome" from chloramphenicol administration led to the concept of the "therapeutic orphan" (Shirkey, 1968), which described the absolute void in the area of pediatric pharmacology in the late 1960's. However, in over 25 years the situation has improved very little. The pharmacologic information that exists for the neonatal population consists largely of anecdotal and case reports or studies comprised of small patient enrollments (Gilman and Gal, 1992). Thus, clinicians are still frequently forced to rely on information and experience derived from adults to formulate drug therapies for children.

The very young infant may be vulnerable to the toxic/adverse effects of xenobiotics by virtue of the biological immaturity of numerous organ systems. This generalized immaturity results in alterations in both the pharmacokinetic and pharmacodynamic profiles of many drugs. While it is possible that these alterations may lead to a reduced toxic potential for a given drug, the converse situation (for example, enhanced toxicity) is a more likely and more serious consequence of physiological immaturity.

Investigation of xenobiotics in developing humans is subject to strict ethical guidelines and restrictions. The issue of informed consent has been codified in such documents as the Helsinki Declaration (Ward and Green, 1988). Informed consent is especially problematic in the case of minor subjects. British and American common law dictates that a guardian can act on behalf of a minor only when that action is in the minor's best interests (Ward

and Green, 1988). The American Academy of Pediatrics Committee on Drugs issued ethical guidelines for the evaluation of drugs in children in 1977 (Gilman and Gal, 1992). These guidelines imply that clinical trials with no direct and immediate therapeutic benefit to the child should not be permitted. The investigation of potentially hazardous entities in the pediatric population is thus largely restricted to appropriate animal models.

A particularly difficult regulatory situation arises when certain "grandfathered" drugs are employed in pediatric patients. These drugs were introduced into medical practice before extensive therapeutic and toxicologic screening was mandatory, and because of a long history of use are classified as "Generally Recognized As Safe" or "GRAS" (Smith and Whyte, 1988; Steinberg, 1993). Chloral hydrate belongs to this category of therapeutic agents. However, the toxic potential of chloral hydrate was recognized shortly after its introduction into medical use. Chloral hydrate was depicted in 1910 as being the most dangerous of all hypnotics (Hoskins, 1984). Children can sustain significant toxic effects after ingestion of as little as 1.5 g of chloral hydrate (Anonymous, 1993; King and England, 1987). Numerous adverse and toxic effects of this agent in the pediatric population have been documented (Dean *et al.*, 1991; Granoff *et al.*, 1971; Greenberg and Faerber, 1990; Hershenson *et al.*, 1984; Hirsch and Zauder, 1986; Lambert *et al.*, 1990; Lansky, 1974; Nordenberg *et al.*, 1971; Reimche *et al.*, 1989; Saarnivaara *et al.*, 1988; Seger and Schwartz, 1994; Silver and Stier, 1971). Because of its "grandfathered" status and the ethical difficulties associated with pediatric drug investigations, the actual toxic potential of chloral hydrate in this vulnerable population remains largely unknown.

2. THE PRESENT INVESTIGATION

2.1 RATIONALE

Chloral hydrate (CH) is the oldest sedative-hypnotic still in clinical use (Butler, 1970; Hoskins, 1984). While once used extensively in almost all patient populations, CH administration is now limited almost exclusively to pediatric and geriatric patients (Cook *et al.*, 1992; Hoskins, 1984; Marx *et al.*, 1993). A 1992 survey of North American pediatric residency programs indicated that CH is still the most commonly used sedative in pediatric practice (Cook *et al.*, 1992).

Despite an extremely long history of use, details of the disposition of CH in the pediatric population were virtually nonexistent until very recently (Gershanik *et al.*, 1981; Gorecki *et al.*, 1990; Hartley *et al.*, 1989; Hindmarsh *et al.*, 1991; Lambert *et al.*, 1990; Laptook and Rosenfeld, 1984; Mayers, 1992; Mayers *et al.*, 1991; Reimche *et al.*, 1989). Both single- and multiple-dose administration have demonstrated significant developmental differences with respect to metabolism and elimination of CH. Of particular interest is the persistence of high levels of potentially toxic metabolites, trichloroacetic acid (TCA) and trichloroethanol (TCE) (Hindmarsh *et al.*, 1991; Mayers, 1992; Mayers *et al.*, 1991).

A number of serious toxic reactions have been attributed to CH ingestion. In particular, CH has been plagued by reports of cardiac arrhythmias, especially in situations of over-dose, in both children and adults (Graham *et al.*, 1988; Gustafson *et al.*, 1977; Hirsch and Zauder, 1986; Marshall, 1977). Few investigations of the cardiotoxic moiety (CH, TCE and/or TCA), the mechanism of arrhythmias, or the susceptibility of neonates to these arrhythmias have been reported (Gross and Hoff, 1968; Riggs *et al.*, 1986; Trulson and Ulissey, 1987; White and Carlson, 1981).

2.2 HYPOTHESIS

It is hypothesized that cardiac arrhythmias induced by CH or its metabolites (TCE and TCA) are analogous to arrhythmias produced by halogenated hydrocarbons such as halothane. It has generally been assumed that halothane cardiotoxicity is due to sensitization of the myocardium to the effects of endogenous catecholamines (Bowyer and Glasser, 1980; Brown and Cade, 1980). However, it has recently been proposed that reductive dehalogenation of halothane, resulting in the formation of radical and carbanion complexes, may cause lipid peroxidation and the subsequent cardiotoxic effects (Gibson and Skett, 1986).

It is proposed that local (cardiac) metabolism of CH results in the formation of reactive species capable of causing lipid peroxidation. This phenomenon would initiate a number of pathologic sequelae which would be responsible for the demonstrated cardiotoxic effects of CH and its metabolites. Susceptibility of the immature myocardium to these arrhythmias may be either higher or lower with respect to the susceptibility of the mature myocardium. It is important to determine whether the neonate is more or less sensitive than the adult to these arrhythmias.

2.3 OBJECTIVES

The objectives of the proposed research were to establish whether:

1. CH or its metabolites are involved in cardiac toxicity or arrhythmogenicity in adult or neonatal rabbit hearts;
2. there are definable levels of CH and its metabolites which produce cardiac arrhythmias in adult and neonatal heart models;
3. the myocardial pharmacokinetics of CH and its metabolites correlate with their cardiotoxic effects;
4. CH and its metabolites are capable of initiating lipid peroxidation in adult and neonatal hearts;
5. myocardial antioxidant enzyme status is associated with either enhanced or diminished CH/metabolite-induced cardiac toxicity.

3. LITERATURE SURVEY

3.1 CHLORAL HYDRATE

3.1.1 Chloral Hydrate Exposure in Humans

3.1.1.1 Therapeutic Use

Chloral hydrate, the first synthetic hypnotic used in medical practice, has enjoyed an extremely long history of therapeutic use. Justus Liebig first synthesized chloral hydrate in 1832 (Alstead, 1936; Butler, 1970; Hoskins, 1984; Sourkes, 1992). Controversy exists regarding the recognition of the hypnotic effects of chloral hydrate - popular opinion attributes the discovery to Oscar Liebreich (1869). However, the pharmacological effect of chloral hydrate had been previously described in 1861 by Rudolf Buchheim (Butler, 1970; Sourkes, 1992). Liebreich was certainly more aware of the therapeutic potential of chloral hydrate, and published a monograph in 1869 enthusiastically recommending its use in various conditions requiring the use of a hypnotic agent (Butler, 1970; Sourkes, 1992).

While once employed extensively in almost all patient populations, chloral hydrate administration is now limited almost exclusively to pediatric and geriatric patients (Cook *et al.*, 1992; Hoskins, 1984; Marx *et al.*, 1993). Chloral hydrate currently has five principal uses in medical practice:

1. treatment of insomnia in adults, primarily geriatric individuals (Hoskins, 1984; Miller and Greenblatt, 1979);
2. treatment of agitation and/or cerebral irritation in neonatal or pediatric patients (American Academy of Pediatrics, 1993; Hartley *et al.*, 1989; Hoskins, 1984; Marx *et al.*, 1993; Mayers *et al.*, 1991; Noerr, 1992);

3. as a sedative for infants and children in ophthalmology and dentistry (Hoskins, 1984; Moore, 1984);
4. as a surgical premedicant to relieve anxiety and facilitate anesthetic induction in young children (Anderson *et al.*, 1990);
5. as a sedative-hypnotic agent in pediatric patients to facilitate non-invasive procedures such as electroencephalography, computed tomography, magnetic resonance imaging (Cook *et al.*, 1992; Green, 1993; Greenberg *et al.*, 1993; Hoskins, 1984; Keeter *et al.*, 1990).

Most pediatric indications for chloral hydrate administration require short-term sedation to allow for the successful completion of diagnostic and therapeutic procedures (American Academy of Pediatrics, 1993). The recommended dose for pediatric sedation is 25-100 mg/kg, with a dose of 50 mg/kg most commonly employed (Cook *et al.*, 1992; Marx *et al.*, 1993). The maximum recommended total dose of chloral hydrate in pediatric patients is 1000-1500 mg, regardless of body weight (Moore, 1984). While chloral hydrate has a long history of safe and effective use for these indications, sedation has proven to be inadequate in 30-40% of patients (Green, 1993). Higher doses (75-100 mg/kg) have been associated with more successful sedation (Greenberg *et al.*, 1993; Lichenstein *et al.*, 1993; Martí-Bonmatí *et al.*, 1995). Some reports indicate that chloral hydrate is less effective for sedation of children over 48 months (Greenberg *et al.*, 1993; Lichenstein *et al.*, 1993) and children with neurologic deficits (Lichenstein *et al.*, 1993; Rumm *et al.*, 1990). Alternative agents for short-term sedation of pediatric patients include the benzodiazepines, barbiturates and phenothiazines (American Academy of Pediatrics, 1993; Green, 1993; Nahata, 1988).

Chloral hydrate is often used in multiple-dosing regimens to maintain prolonged sedation for critically ill neonatal patients undergoing mechanical ventilation (American Academy of Pediatrics, 1993; Goldsmith, 1994; Hartley *et al.*, 1989; Laptook and Rosenfeld, 1984; Mayers *et al.*, 1991; Noerr, 1992). The two most common indications for the use of chloral hydrate sedation in neonatal patients are to prevent agitated patients from "fighting the ventilator"

and in chronic cases that involve bronchopulmonary dysplasia (Goldsmith, 1994). This approach involves the administration of chloral hydrate every 4 to 6 hours over a period of days to weeks (Hartley *et al.*, 1989). It is recommended that this type of subchronic multiple dosing regimen with chloral hydrate be approached with caution because signs of toxicity may appear to be nonspecific and may not be apparent for some hours after drug administration (Laptook and Rosenfeld, 1984). Neonates, especially those who are critically ill and/or premature, may be predisposed to the toxic effects of chloral hydrate by virtue of their immature elimination processes; therefore, close monitoring of infants maintained on chloral hydrate sedation for prolonged periods of time has been recommended (Gershanik *et al.*, 1981; Goldsmith, 1994; Hartley *et al.*, 1989; Laptook and Rosenfeld, 1984; Noerr, 1992).

Several recent studies have detailed the pharmacokinetic and pharmacodynamic aspects of chloral hydrate administration in the pediatric population after both single- and multiple-dosing regimens (Gershanik *et al.*, 1981; Gorecki *et al.*, 1990; Hartley *et al.*, 1989; Hindmarsh *et al.*, 1991; Lambert *et al.*, 1990; Laptook and Rosenfeld, 1984; Mayers, 1992; Mayers *et al.*, 1991; Reimche *et al.*, 1989). Specific information on the pharmacology of chloral hydrate in pediatric patients has greatly assisted in the rational use of this agent.

3.1.1.2 Environmental Exposure

While therapeutic administration is the most obvious source of chloral hydrate ingestion, significant exposure to chloral hydrate and its metabolites can occur from environmental contamination. Chloral hydrate has been identified as a by-product of such chlorination processes as the disinfection of drinking water and the bleaching softwood pulp (Daniel *et al.*, 1992a; Daniel *et al.*, 1992b; Hrudey *et al.*, 1995; Klinefelter *et al.*, 1995; Larson and Bull, 1992a; Sanders *et al.*, 1982; Steinberg and DeSesso, 1993; U.S. Environmental Protection Agency, 1975; U.S. Environmental Protection Agency, 1982; Uden

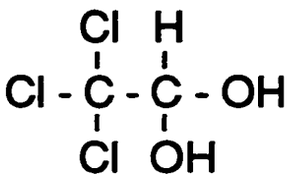
and Miller, 1983). Industrial use of chloral hydrate as an intermediate in the production of pesticides and plastics also contributes to drinking water contamination (Daniel *et al.*, 1992a; Sanders *et al.*, 1982). The United States Environmental Protection Agency has measured chloral hydrate in municipal water supplies at levels of 2-5 µg/litre (National Academy of Sciences, 1977; U.S. Environmental Protection Agency, 1975). It has been suggested that trichloroacetic acid, one of the principle metabolites of chloral hydrate, is the major nonvolatile contaminant produced by water chlorination (Larson and Bull, 1992a). Trichloroacetic acid concentrations in municipal water supplies have been reported as ranging between 30-160 µg/ml (Uden and Miller, 1983). The suggested safe level of trichloroacetic acid in drinking water has been estimated as 0.175 µg/ml (Steinberg and DeSesso, 1993).

Environmental exposure to chloral hydrate may also be secondary to trichloroethylene exposure, with subsequent metabolic conversion of trichloroethylene to chloral hydrate (Dekant *et al.*, 1984; Green and Prout, 1985; Ikeda *et al.*, 1980; Miller and Guengerich, 1983; Müller *et al.*, 1974; Müller *et al.*, 1982; Sanders *et al.*, 1982; Steinberg and DeSesso, 1993). Trichloroethylene has been extensively employed in many industrial applications as a solvent (Dekant *et al.*, 1984; Müller *et al.*, 1982; Saillenfait, 1995; Steinberg and DeSesso, 1993). Exposure to trichloroethylene results from both contamination in the workplace as well as ground and surface water pollution (Larson and Bull, 1992b; Saillenfait, 1995; Steinberg and DeSesso, 1993). The United States currently limits trichloroethylene content of potable water to 5 µg/litre, whereas Canadian drinking water guidelines are 50 µg/litre (Steinberg and DeSesso, 1993). However, the most significant source of exposure in urban areas is from the inhalation of ambient of atmospheric trichloroethylene (Steinberg and DeSesso, 1993). It has been estimated that approximately 60% of the total global production of trichloroethylene is released into the atmosphere annually (Müller *et al.*, 1982).

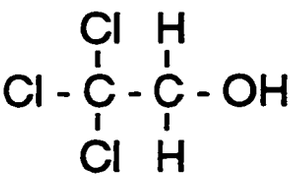
3.1.2 Elimination

3.1.2.1 Mature Animals

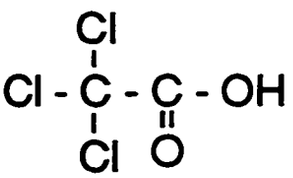
Chloral hydrate is rapidly metabolized *in vivo* both by reduction to trichloroethanol (TCE) and by oxidation to trichloroacetic acid (TCA) (Figure 3-1). When administered to healthy adult volunteers, CH is nearly undetectable within minutes of administration (Breimer *et al.*, 1974; Gorecki *et al.*, 1990).



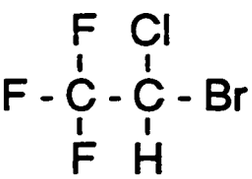
Chloral hydrate



Trichloroethanol



Trichloroacetic acid



Halothane

Figure 3-1. Chemical structures for chloral hydrate, trichloroethanol, trichloroacetic acid and halothane.

Numerous enzyme/cofactor systems have been proposed as playing important roles in the metabolism of chloral hydrate (Friedman and Cooper, 1960); however, more recent studies have demonstrated that the reduction of CH to TCE occurs primarily at extra-hepatic sites, and is catalyzed by

aldehyde reductase (EC 1.1.1.2). This reductive reaction employs NADPH as a cofactor (Hobara *et al.*, 1987; Ikeda *et al.*, 1981; Ikeda *et al.*, 1980; Shultz and Weiner, 1979; Tabakoff *et al.*, 1974). Alcohol dehydrogenase (EC 1.1.1.1) is capable of catalyzing the reduction of CH to TCE; however, this reaction occurs *in vivo* only with extremely high levels of NADH (Shultz and Weiner, 1979). While small amounts of TCE are excreted unchanged in the urine, most of the TCE metabolite undergoes conjugation with glucuronic acid prior to excretion in the urine (Breimer *et al.*, 1974; Ikeda *et al.*, 1984; Sellers *et al.*, 1978). TCE-glucuronide is excreted in the bile (approximately 5% of an intravenous dose); however, the absence of TCE or its glucuronide in the feces has led to the speculation that extensive biliary recycling of the TCE-glucuronide metabolite occurs (Garrett and Lambert, 1973).

An NAD-dependent aldehyde dehydrogenase (EC 1.2.1.3) is believed to be primarily responsible for the hepatic oxidation of CH to TCA (Berger and Weiner, 1977; Breimer *et al.*, 1974). Aldehyde dehydrogenase is also located in extra-hepatic tissues, such as the erythrocyte, brain and heart, and may contribute to the formation of TCA in these extra-hepatic sites (Berger and Weiner, 1977; Goedde and Agarwal, 1990; Inoue *et al.*, 1979). TCA can also be formed directly from TCE in a reaction catalyzed by mixed-function oxidases, possibly employing CH as an intermediary (Breimer *et al.*, 1974; Kawamoto *et al.*, 1987; Marshall and Owens, 1954). TCA is eventually excreted unchanged in the urine (Breimer *et al.*, 1974; Sellers *et al.*, 1978). *In vivo* studies in both rats and mice have demonstrated biliary excretion of TCA, suggesting the possibility of hepatic conjugation of this metabolite (Green and Prout, 1985).

An alternative scheme for the metabolism of CH proposes that a carbonyl reductase enzyme (EC 1.1.1.184) catalyzes an aldehyde dismutation reaction with redox cycling of NAD(P) cofactors. Carbonyl reductase activity has been detected in most mammalian tissues, and is believed to be important in the detoxification of xenobiotics and in the reductive metabolism of carbonyl compounds derived from lipid peroxidation. The enzyme apparently oxidizes

chloral hydrate to TCA using $NAD(P)^+$ as the cofactor and reduces chloral (the unhydrated aldehyde form) to TCE with NAD(P)H. This reaction produces equimolar amounts of TCE and TCA with little alteration of cofactor levels. The affinity of carbonyl reductase for CH is higher than that of aldehyde reductase, leading to speculation that carbonyl reductase acts as a major CH-metabolizing enzyme (Hara *et al.*, 1991). Figure 3-2 illustrates a metabolic pathway for chloral hydrate which encompasses the existing evidence regarding its metabolic fate.

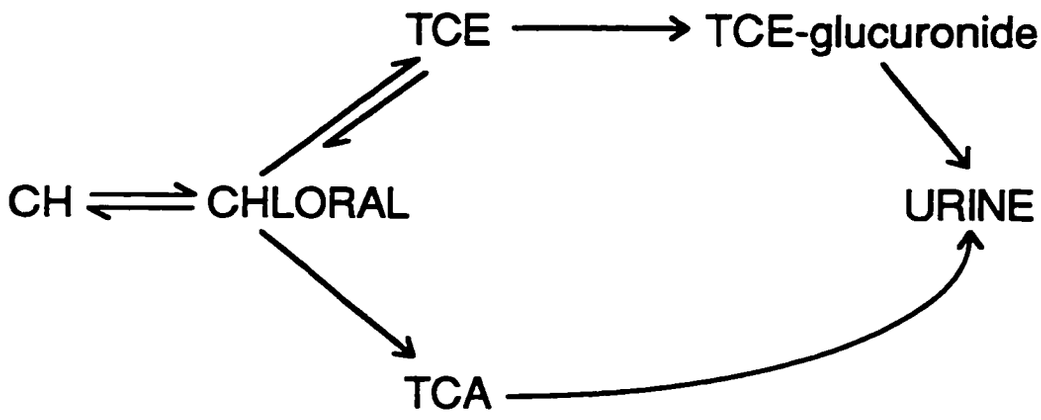


Figure 3-2. A possible pathway for the *in vivo* biotransformation of chloral hydrate.

The elimination half-life of TCE is approximately 8-12 hours in the adult (Breimer *et al.*, 1974; Sellers *et al.*, 1978), and the half-life of the glucuronide conjugate is about 6-8 hours (Breimer *et al.*, 1974). TCA has an extremely long half-life, which has been reported to be between 67 and 100 hours (Breimer *et al.*, 1974; Sellers *et al.*, 1972b; Sellers *et al.*, 1978). It has been hypothesized that extensive tissue binding of TCA (Garrett and Lambert, 1973; Müller *et al.*, 1974) and/or biliary recycling of a TCA-glucuronic acid conjugate (Green and Prout, 1985) may account for its very long half-life. The persistence of TCA has led to concern regarding its toxic potential (Hindmarsh *et al.*, 1991;

Kawamoto *et al.*, 1987; Müller *et al.*, 1974; Sellers *et al.*, 1978; Steinberg, 1993).

3.1.2.2 Immature Animals

Elimination of CH in the neonate is characterized by immaturity of elimination processes. The activities of most metabolic processes are markedly depressed for at least two weeks after birth (Morselli, 1989; Reed and Besunder, 1989). While oxidative capacity of the human newborn is substantially less than that in the adult, significant levels of xenobiotic oxidation do occur (Morselli, 1989; Reed and Besunder, 1989). For example, aldehyde dehydrogenase activity in the fetus is roughly 10-20% of adult levels (Perucca, 1987). Two alcohol dehydrogenase isozymes have been detected in the neonate, with full adult activity being achieved at about five years of age (Perucca, 1987). Human neonates have cytochrome P450-dependent reductase activity equivalent to about one-half that of adults (Juchau, 1990). In contrast to a relatively well-developed oxidative system in the newborn, phase II conjugation reactions, especially glucuronidation, display very low activities (Besunder *et al.*, 1988; Kandall *et al.*, 1973; Pacifici *et al.*, 1990; Radde, 1985a). Xenobiotics which rely primarily on glucuronidation for elimination are potentially toxic in neonates because of the compromised activity of this pathway and because alternate pathways for detoxification and elimination are generally not adequate (Milsap and Szefler, 1987).

The relative metabolic immaturity observed in neonates would be expected to alter the metabolic fate of CH in this population. However, despite an extremely long history of use, details of the disposition of CH in the pediatric population were virtually nonexistent until very recently (Gershanik *et al.*, 1981; Gorecki *et al.*, 1990; Hartley *et al.*, 1989; Hindmarsh *et al.*, 1991; Lambert *et al.*, 1990; Laptook and Rosenfeld, 1984; Mayers, 1992; Mayers *et al.*, 1991; Reimche *et al.*, 1989). Contrary to the situation in the adult, CH was detectable for several hours after a single oral dose (50 mg/kg) in neonates

and young children (Mayers *et al.*, 1991). The persistence of the parent compound is indicative of reduced metabolic elimination of CH.

Renal elimination processes are also incompletely developed in human neonates. Both glomerular filtration and tubular secretion are less efficient in immature kidneys (Besunder *et al.*, 1988; Kearns and Reed, 1989; Radde, 1985b). Therefore, when drugs which are eliminated primarily by renal excretion are administered to neonatal subjects they exhibit lower body clearances and longer elimination half-lives. There is evidence of diminished elimination of TCE and TCA due to immature renal excretory mechanisms. The elimination half-lives of both TCE (30-40 h) (Gershanik *et al.*, 1981; Hindmarsh *et al.*, 1991; Mayers *et al.*, 1991) and TCA (>6 days) (Hindmarsh *et al.*, 1991; Mayers *et al.*, 1991) are substantially prolonged in neonates when compared to adult volunteers (8-12 h and 67-100 h, respectively) (Breimer *et al.*, 1974; Sellers *et al.*, 1972b; Sellers *et al.*, 1978).

The general physiologic immaturity exhibited by human neonates results in a situation with potential toxicologic significance when chloral hydrate is administered in this population. It has been established that neonates and young children tend to accumulate TCE and TCA, especially in multiple-dosing situations (Hindmarsh *et al.*, 1991; Reimche *et al.*, 1989). Of particular concern in the neonatal population is the persistence of TCA, a potentially toxic metabolite, even after a single oral dose (Hindmarsh *et al.*, 1991; Mayers *et al.*, 1991).

3.1.2.3 Metabolic Activation of Halogen-Containing Compounds

Numerous therapeutic agents rely on the cytochrome P450-dependent microsomal drug biotransformation system for elimination. The cytochrome P450 (P450) enzyme superfamily is an ubiquitous mixed-function oxidase system located in both hepatic and extra-hepatic tissues (George and Farrell, 1991; Parke *et al.*, 1990). All of the P450 enzymes function as monooxygenases, but they also have the potential to act as oxidases,

peroxidases and reductases, depending upon the particular reaction environment involved (Parke *et al.*, 1990; Vaz *et al.*, 1988).

While enzyme-catalyzed metabolism of xenobiotics is usually associated with detoxification and accelerated elimination, the converse situation (i.e. enhanced toxicity) may also occur, resulting in the formation of reactive intermediates. Indirect evidence suggests the generation of free radical intermediates at several stages of the catalytic cycle of cytochrome P450 (George and Farrell, 1991; Vaz *et al.*, 1988). Quinone production, reduction and dehalogenation reactions have been especially associated with the formation of reactive radical species (Angus *et al.*, 1990; Parke *et al.*, 1990).

Cytochrome P450-mediated reactions have been implicated in the metabolic activation of CH and its metabolites (Larson and Bull, 1992a; Ni *et al.*, 1996). Specifically, cytochrome P450IIE1 (CYP2E1) is believed to be the major enzyme which catalyzes the metabolic activation of CH, TCE and TCA in an *in vitro* hepatic microsomal system (Ni *et al.*, 1996). Figure 3-3 illustrates the metabolic scheme proposed to describe the CYP2E1-catalyzed formation of reactive intermediates of chloral hydrate (Ni *et al.*, 1996). The authors believe that the primary route for radical formation seems to be via the formation of TCA from CH, although other activation pathways resulting in the formation of different radical species may also be involved (Ni *et al.*, 1996). The CYP2E isozyme is known to readily generate oxygen free radical species by oxygenating substrates (i.e. ethanol) by a nonenzymatic radical mechanism rather than by direct insertion of active oxygen from the cytochrome (George and Farrell, 1991; Parke *et al.*, 1990; Vaz *et al.*, 1988). The potential of CYP2E1 to form free radical species is greater than that of any other P450 protein (George and Farrell, 1991). It is known that CYP2E1 is the primary isozyme responsible for the biotransformation of other low molecular weight halogen-containing chemicals, such as carbon tetrachloride, chloroform, 1,1,1-trichloroethane and trichloroethylene (Ni *et al.*, 1996). There is a very high degree of functional homology of CYP2E1 proteins between species, lending

credence to the extrapolation of the results of *in vivo* toxicity studies in experimental animals to humans (George and Farrell, 1991).

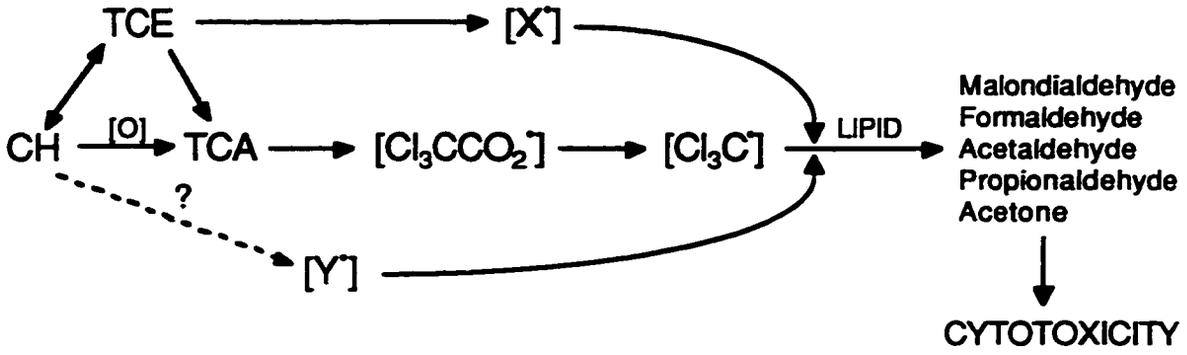


Figure 3-3. Metabolic scheme proposed to describe the CYPIIE1-catalyzed formation of reactive intermediates of chloral hydrate (adapted from Ni *et al.*, 1996).

Experimental evidence suggests that xenobiotic metabolism may be profoundly affected by tissue oxygenation (Angus *et al.*, 1990). Oxygen is a substrate for P450 during oxidation reactions, and cellular redox state, as measured by the NAD(P)H:NAD(P)$^+$ ratio, is an important determinant of numerous drug biotransformations (Angus *et al.*, 1990). A high NAD(P)H:NAD(P)$^+$ ratio is indicative of cellular hypoxia, and would be predicted to favor reductive metabolic reactions during anaerobic conditions (Angus *et al.*, 1990; Gibson and Skett, 1986). The formation of reductive metabolites is believed to be important in the cytotoxicity of a number of chemical agents (Angus *et al.*, 1990; Dargel, 1992; Gibson and Skett, 1986). There appears to be a link between the electrophilicity of halogenated hydrocarbon compounds and their propensity to form free radical intermediates through reductive biotransformation (Crebelli *et al.*, 1995). A metabolic scheme showing the reductive dehalogenation of TCA with subsequent oxidative peroxyl radical formation has been proposed (Larson

and Bull, 1992a). The first step in the metabolism of TCA could be a P450-mediated one electron reduction and homolytic cleavage yielding the dichloroacetyl (DCA) radical (Figure 3-4). The DCA radical could subsequently abstract a hydrogen from lipid yielding DCA, or could form the dichloroacetylperoxyl radical following reaction with molecular oxygen (Larson and Bull, 1992a). Halogenated peroxyl radicals are very good oxidants, and are believed to be responsible for tissue injury *in vivo* (Asmus *et al.*, 1988).

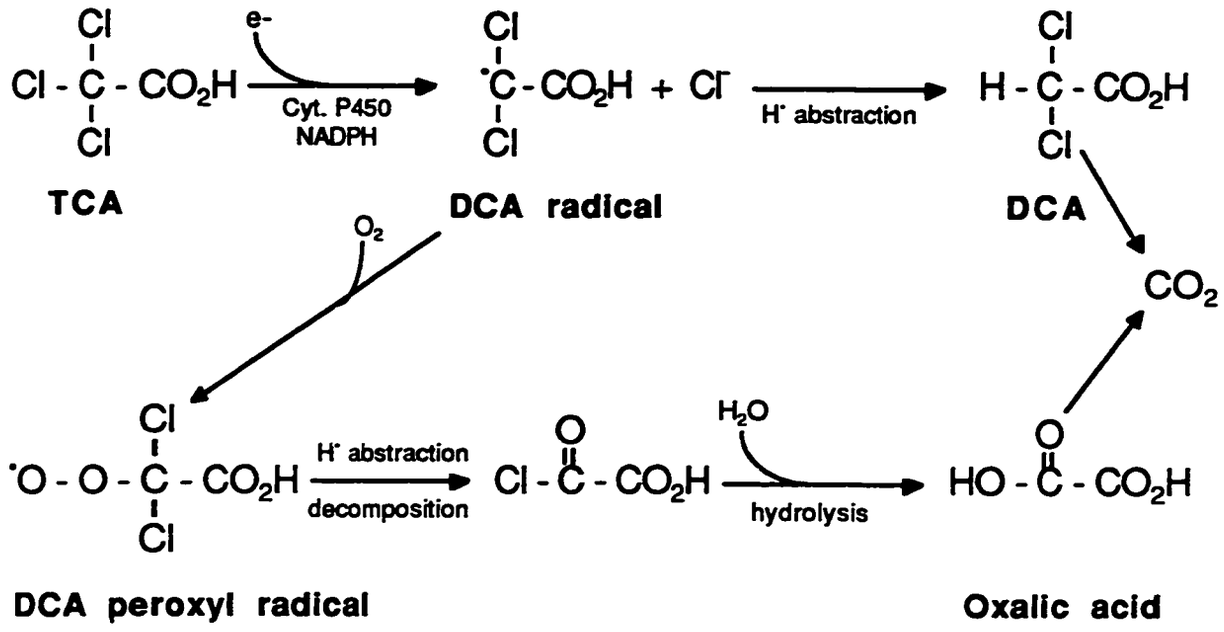


Figure 3-4. Metabolic scheme illustrating the reductive dehalogenation of trichloroacetic acid and oxidative peroxyl radical formation (adapted from Larson and Bull, 1992). TCA=trichloroacetic acid; DCA=dichloroacetic acid

Thus, metabolic activation of CH and its metabolites can occur by either oxidative or reductive mechanisms, resulting in radical species capable of causing tissue injury *in vivo*.

3.1.3 Toxic Effects

3.1.3.1 Introduction

Chloral hydrate belongs to the group of "grandfathered" drugs for which the current standard of evaluation has not been met due to a long history of medical use (Smith and Whyte, 1988). These drugs fall into the category "Generally Recognized As Safe" or "GRAS" (Steinberg, 1993). However, the toxic potential of chloral hydrate was recognized shortly after its introduction into medical use. Leibreich himself described both acute and chronic chloral hydrate-related fatalities as early as 1871. Forty-four chloral hydrate-induced deaths were described in 1890, of which 22 occurred between 1871 and 1872. Chloral hydrate was depicted in 1910 as being the most dangerous of all hypnotics (Hoskins, 1984). Clinicians were not, however, unanimous in the condemnation of chloral hydrate (Alstead, 1936).

3.1.3.2 Adverse Effects

Chloral hydrate is generally believed to have a wide margin of safety in both pediatric and adult medicine, especially when administered for acute indications (Hoskins, 1984; Moore, 1984). An epidemiological study of 1618 patients in 1969 revealed a low incidence of adverse events after administration of chloral hydrate. Serious reactions proved to be rare, and all adverse effects were resolved completely (Hoskins, 1984). A more recent study of the adverse effects of chloral hydrate administration in 5435 adult patients also demonstrated a very low occurrence of adverse reactions (Miller and Greenblatt, 1979). These authors determined that undesirable CNS depression was the most prevalent adverse reaction (1.1%). The next most common adverse reactions were sensitivity reactions (0.3%) and gastrointestinal disturbances (0.3%). Paradoxical CNS excitation, headache, hepatic decompensation, coagulation disturbances and various cardiovascular complaints were encountered rarely. Only 3 patients exhibited

life-threatening reactions which could be at least partially attributed to chloral hydrate ingestion, all of whom recovered fully (Miller and Greenblatt, 1979).

A clinical evaluation of the use of chloral hydrate in pediatric dentistry found doses in the 40-60 mg/kg range to be relatively safe and effective. The main risks associated with recommended doses of chloral hydrate appeared to be minimal, and included tissue irritability, gastric upset and over-sedation. Dose related reactions such as prolonged CNS depression and vomiting seemed to be more prevalent in pediatric patients. Several factors were suggested to increase the risk of using chloral hydrate in the pediatric population: age (<2 years) and weight (<35 pounds); dose (>40-60 mg/kg); drug combinations (barbiturates, alcohol, nitrous oxide or benzodiazepines); and physical status (marked renal, hepatic or cardiac impairment) (Moore, 1984). Another pediatric study of 140 children (age 0-36 months) given chloral hydrate (average dose 87 mg/kg) as an oral premedicant prior to echocardiography revealed that 18% of patients experienced paradoxical excitement before sedation occurred, and all patients demonstrated motor and affective changes after sedation. The authors concluded, however, that chloral hydrate was a safe and effective pediatric sedative agent (Lipshitz *et al.*, 1993). Of 131 pediatric patients undergoing MRI or CT scans who were administered chloral hydrate as a premedicant, 19% experienced excessive sedation and 3% exhibited agitation (Merola *et al.*, 1995). Acute administration of chloral hydrate in pediatric patients appears to be both safe and effective, with minor adverse reactions being relatively infrequent.

3.1.3.3 Drug Interactions

Chloral hydrate has been implicated in numerous drug interactions. The most frequently reported interactions involve additive CNS depressant effects when chloral hydrate is co-administered with other sedative agents (Moore, 1984). This effect has been exploited in clinical situations to allow for a reduction in the doses of both chloral hydrate and promethazine, thereby minimizing the occurrence of nausea and vomiting experienced with either

drug given alone (Moore, 1984). Serious CNS depression can occur, however, and close supervision of patients is recommended when chloral hydrate is administered with other CNS depressant agents (Moore, 1984).

The notorious combination of chloral hydrate with ethanol is thought to cause a supra-additive drug interaction (Moore, 1984). This effect - referred to as the "Mickey Finn" - is reputed to cause an enhanced CNS depressant effect of ethanol, along with tachycardia, facial flushing and headache in some cases (Sellers *et al.*, 1972a). It has been suggested that the metabolic conversion of chloral hydrate to trichloroethanol (the putative CNS-active metabolite) is enhanced by ethanol, while trichloroethanol competitively inhibits the metabolism of ethanol by alcohol dehydrogenase (Moore, 1984; Shultz and Weiner, 1979). However, an immediate "knock-out" effect of chloral hydrate and ethanol co-administration has not been demonstrated in controlled scientific studies in man (Sellers *et al.*, 1972a).

Trichloroacetic acid has long been implicated in drug interactions involving drug displacement from binding to albumin. The activities of oral anticoagulants bis-hydroxycoumarin and sodium warfarin are affected by chloral hydrate administration (Moore, 1984). The activity of bis-hydroxycoumarin is apparently inhibited after repeated doses of chloral hydrate, possibly resulting from reduced plasma concentrations of the anticoagulant (Breimer, 1977; Moore, 1984). Sodium warfarin appears to be displaced from serum binding sites after chloral hydrate administration, causing excessive hypoprothrombinemia (Breimer, 1977; Moore, 1984). While chloral hydrate should be used with caution in patients taking oral anticoagulant agents, it is believed that the interactions may be clinically insignificant, especially with single dose administration of chloral hydrate (Hoskins, 1984; Moore, 1984).

An interaction has also been reported between chloral hydrate and furosemide, which is characterized by transient diaphoresis, hot flashes, variable blood pressure, tachycardia and hypertension (Dean *et al.*, 1991; Malach and Berman, 1975). This reaction is said to occur when an

intravenous bolus dose of furosemide is preceded 8-24 hours by chloral hydrate (Moore, 1984). This reaction is believed to involve the displacement of trichloroacetic acid from plasma protein binding sites by furosemide, followed by displacement of protein bound thyroxin by free trichloroacetic acid (Malach and Berman, 1975). However, a recent study of the protein binding characteristics of trichloroacetic acid in adult, neonatal cord and neonatal exchange transfusion plasma showed that clinically relevant concentrations of furosemide did not affect the plasma protein binding of trichloroacetic acid (Mayers, 1992).

Chloral hydrate administration has been reported to reduce the *in vivo* plasma protein binding of sulfamethoxazole (Imamura *et al.*, 1984). This interaction is believed to proceed indirectly through the formation of trichloroacetic acid. Trichloroacetic acid directly displaces sulfamethoxazole from binding sites, while the increased availability of the parent drug to drug metabolizing enzymes results in enhanced metabolite formation, which also competes with sulfamethoxazole for plasma protein binding sites.

Chloral hydrate administration in neonatal patients has also been associated with the development of direct (unconjugated) (Lambert *et al.*, 1990) and indirect (conjugated) (Reimche *et al.*, 1989) hyperbilirubinemia. Displacement of conjugated bilirubin from plasma protein binding sites by trichloroacetic acid may produce direct hyperbilirubinemia (Lambert *et al.*, 1990; Onks *et al.*, 1992). Indirect hyperbilirubinemia may result from competitive inhibition of bilirubin conjugation with glucuronic acid by trichloroethanol (Onks *et al.*, 1992; Reimche *et al.*, 1989). It is suggested that prolonged chloral hydrate administration in critically ill neonates should be approached with caution to avoid the development of jaundice or kernicterus (Lambert *et al.*, 1990; Onks *et al.*, 1992; Reimche *et al.*, 1989).

Several reports have implicated chloral hydrate in the competitive inhibition of various aldehyde dehydrogenase isozymes (Berger and Weiner, 1977; Inoue *et al.*, 1979; Maki and Sladek, 1993; Sharpe and Carter, 1993). Aldehyde dehydrogenase catalyzes the oxidative biotransformation of

aldehydes to acids, and is located in the liver, erythrocytes, kidney, intestine, stomach, brain and heart (Berger and Weiner, 1977; Goedde and Agarwal, 1990; Inoue *et al.*, 1979). Acetaldehyde is one of the most important substrates for this reaction (Berger and Weiner, 1977; Goedde and Agarwal, 1990). The aldehyde substrates are formed from the metabolism of ingested alcohols such as ethanol, or from endogenous amines like serotonin (Berger and Weiner, 1977; Goedde and Agarwal, 1990). Significant amounts of various aldehydes, including acetaldehyde, are also formed during lipid peroxidation of cellular membranes (Goedde and Agarwal, 1990). Biogenic aldehydes are known to be highly reactive substances as a result of their carbonyl group (Goedde and Agarwal, 1990). For example, acetaldehyde covalently binds to numerous proteins resulting in altered organ structure and function (Goedde and Agarwal, 1990). Therefore, efficient detoxification of these compounds is a requirement for the maintenance of normal cell function (Goedde and Agarwal, 1990). The inhibition of aldehyde dehydrogenase by chloral hydrate is of particular concern since chloral hydrate is utilized therapeutically in the treatment of withdrawal syndromes associated with certain drugs and insomnia in alcoholic patients (Berger and Weiner, 1977). Inhibition of acetaldehyde metabolism may be responsible for many of the peripheral symptoms of the ethanol/chloral hydrate interaction (facial flushing, tachycardia, palpitation and headache) (Goedde and Agarwal, 1990; Maki and Sladek, 1993).

3.1.3.4 Caustic Reactions

Both chloral hydrate and trichloroacetic acid are caustic in nature (Windholz, 1983), and have been demonstrated to be skin and mucous membrane irritants in humans (Ganepola, 1992; Granoff *et al.*, 1971; Rall, 1990). Irritation of the mouth, edema of the larynx, ulceration and strictures of the esophagus and ulceration of the stomach, as well as corrosive burns to the face, mouth, pharynx and larynx have been reported (Ganepola, 1992). Chloral hydrate administration commonly causes gastric irritation when

administered orally (Miller and Greenblatt, 1979; Silverman and Muir, 1993). General visceral inflammatory reactions with intra-peritoneal administration of chloral hydrate in animals have been documented. These reactions include adynamic ileus, ileitis, intestinal obstruction, peritonitis and gastric ulcers (Anyebuno and Rosenfeld, 1991; Silverman and Muir, 1993). A case report of neonatal intoxication attributed the occurrence of adynamic ileus and bladder atony to chloral hydrate administration. These authors, however, suggest parasympathetic inhibition or sympathetic potentiation as the toxic mechanism (Anyebuno and Rosenfeld, 1991).

3.1.3.5 Acute Systemic Toxicity

The vast majority of acute toxic reactions to chloral hydrate have occurred as a result of deliberate or inadvertent overdose (Moore, 1984). Chloral hydrate fatalities are still reported for both adult and pediatric patients. The acute toxic dose of chloral hydrate in adults is believed to be 10 g (Rall, 1990); however, the range of doses associated with chloral hydrate toxicity is quite wide. In adults, as little as 3-4 g has resulted in death, whereas patients have survived after doses as large as 50 g (Baselt *et al.*, 1975; King and England, 1987). Children can sustain significant toxic effects after ingestion of as little as three capsules (1.5 g) (Anonymous, 1993; King and England, 1987), while an ingestion of 8 g in a six-year-old child caused no untoward effects (Mindham, 1968). Since the half-life of chloral hydrate is very short, trichloroethanol levels are commonly measured in suspected chloral hydrate intoxication. The toxic blood concentration of trichloroethanol is reported to be >100 µg/ml (range 100-640 µg/ml) (Baselt *et al.*, 1975). Fatal chloral hydrate intoxications have resulted in trichloroethanol blood concentrations of 32.6-127 µg/ml (Heller *et al.*, 1992; Jastak and Pallasch, 1988; Jindrich, 1977; Meyer *et al.*, 1995).

Acute chloral hydrate intoxication is characterized by depression of the central nervous, respiratory and cardiovascular systems (Hoskins, 1984). A typical clinical scenario of chloral hydrate overdose would include coma,

hypotension, and hypoventilation, with supraventricular and ventricular arrhythmias frequently present (Hoskins, 1984; Moore, 1984). Death after a fatal dose of chloral hydrate usually results from resistant cardiac arrhythmias (Graham *et al.*, 1988). The recommended approach to chloral hydrate overdose is symptomatic treatment, and hemodialysis/hemoperfusion (Hoskins, 1984; Moore, 1984), or exchange transfusion in neonatal patients (Anyebuno and Rosenfeld, 1991).

3.1.3.5.1 Central Nervous System Toxicity

A retrospective study of 5435 medical patients who received chloral hydrate in the course of their hospital stay cited central nervous system (CNS) depression as the most frequently reported adverse reaction in this population. Undesirable CNS depression was more common with increasing dosage in older (>50 years) patients, in patients who died during hospitalization, in patients who were co-administered benzodiazepine anxiolytics and in patients whose blood urea nitrogen level was elevated (Miller and Greenblatt, 1979). The central-depressant effects of chloral hydrate have generally been attributed to its reduced metabolite trichloroethanol (Marshall and Owens, 1954; Rall, 1990). However, a recent investigation of the sedative/hypnotic effects of chloral hydrate in neonates suggests that the parent drug may play an important therapeutic role in this population. It is possible that chloral hydrate induces the immediate sedative effects, with trichloroethanol providing the prolonged sedation observed in some patients (Mayers *et al.*, 1992).

The CNS depressant effects of chloral hydrate are dose-dependent, with more pronounced depression progressing from relaxation, lethargy, drowsiness and hypnosis to loss of consciousness and coma (Moore, 1984). Symptoms of toxic-metabolic encephalopathy induced by chloral hydrate include: miosis with conservation of pupillary reflexes; reduced or absent oculocephalic responses; absence of oculovestibular (caloric) responses; loss of deep-tendon reflexes; and muscle flacidity (Lansky, 1974). In most clinical situations the coma produced by inadvertent or deliberate overdose of chloral

hydrate resolves upon withdrawal of the drug (Lansky, 1974; Laptook and Rosenfeld, 1984; Miller and Greenblatt, 1979).

Paradoxical central nervous excitation reactions have also been observed with chloral hydrate use. These reactions typically manifest as agitation, restlessness, hallucination, nervousness, hyperactivity or nightmares (Miller and Greenblatt, 1979). Some patients display a rare idiosyncratic response, in which they may become disoriented, incoherent and possibly exhibit paranoid behavior (Rall, 1990). While it is believed that the CNS excitation reactions occur primarily in older patients (Miller and Greenblatt, 1979), similar responses have been observed in pediatric patients (Lipshitz *et al.*, 1993; Merola *et al.*, 1995).

3.1.3.5.2 Respiratory Toxicity

Chloral hydrate is a popular sedative in studies of respiratory mechanics because of its apparent lack of effect on any respiratory parameter (Biban *et al.*, 1993; Rall, 1990). A systematic investigation of the effects of chloral hydrate on the ventilatory response to hypercarbia or hypoxia in unanesthetized adult rabbits determined that chloral hydrate had no effect on chemical inspiratory drive (Hunt *et al.*, 1982). Chloral hydrate has been reported, however, to cause respiratory depression in infants recovering from acute viral bronchiolitis (Biban *et al.*, 1993). Two cases of respiratory insufficiency in children with Leigh's syndrome (subacute necrotizing encephalomyelopathy) were attributed to a further depression at the level of the brainstem caused by chloral hydrate (Greenberg and Faerber, 1990).

Several reports of adverse respiratory events occurring in pediatric patients predisposed to obstructive sleep apnea after therapeutic doses of chloral hydrate have appeared in the literature. A 22-month-old infant experienced a near-fatal case of acute obstruction of the upper airway immediately following administration of chloral hydrate elixir. The presumed cause of the obstruction in this case was acute laryngospasm, secondary to chloral hydrate aspiration (Granoff *et al.*, 1971). A second case involved a

three-year-old child who experienced obstructive sleep apnea and a near-fatal airway obstruction followed by respiratory arrest after chloral hydrate administration (Hershenson *et al.*, 1984). An *in vivo* investigation in cats and rabbits was subsequently carried out to determine the possible mechanism of the chloral hydrate-associated airway obstruction in this case. The authors reported that chloral hydrate at normal hypnotic doses may preferentially depress genioglossus activity as compared with diaphragmatic activity, thereby predisposing the subject to serious airway obstruction due to a failure of the airway maintaining musculature (Hershenson *et al.*, 1984). Two further cases of respiratory failure after chloral hydrate administration in two 24-month-old children with suspected obstructive sleep apnea have been reported (Biban *et al.*, 1993). Chloral hydrate was believed to contribute to depression of airway maintaining muscles and impairment of related compensatory mechanisms in these cases. The authors conclude that children affected by obstructive sleep apnea could experience life-threatening respiratory obstruction following sedation with chloral hydrate; therefore, continuous monitoring of vital parameters and oxygen saturation is recommended during sedation (Biban *et al.*, 1993).

3.1.3.5.3 Cardiovascular Toxicity

Chloral hydrate ingestion has long been associated with the development of arrhythmias, especially in cases of overdose (Bowyer and Glasser, 1980; Brown and Cade, 1980; Byatt and Volans, 1984; DiGiovanni, 1969; Gleich *et al.*, 1967; Graham *et al.*, 1988; Gross and Hoff, 1968; Gustafson *et al.*, 1977; Hirsch and Zauder, 1986; Jastak and Pallasch, 1988; Laptook and Rosenfeld, 1984; Marshall, 1977; Nordenberg *et al.*, 1971; Silver and Stier, 1971; Stalker *et al.*, 1978; Vaziri *et al.*, 1977; Wiseman and Hampel, 1978; Young *et al.*, 1986). A report published in 1936 sought to address the potential for cardiovascular toxicity with the use of chloral hydrate (Alstead, 1936). The author of that study concluded: "Chloral hydrate in therapeutic doses has no harmful effect upon the heart" (Alstead, 1936). The lack of a

control group and a questionable experimental design limit the value of this early investigation. Tables 3-1 and 3-2 describe reports of chloral hydrate-related cardiac toxicity in pediatrics and adults, respectively. It has been estimated that approximately 25% of chloral hydrate intoxications will result in extrasystoles (Wiseman and Hampel, 1978).

Table 3-1. Chloral hydrate-associated cardiotoxicity reported in pediatric subjects (adapted from Mayers, 1992).

DOSE	AGE	SEX	CARDIAC SYMPTOMS	REFERENCE
1.5 g	28 mo	M	PVC	Nordenberg et al., 1971
96 mg/kg/d	9 y	F	SVT	Hirsch & Zauder, 1986
1.4 g	30 mo	F	SVT	Hirsch & Zauder, 1986
≤500 mg/kg/d	10 mo	M	SVT	Hirsch & Zauder, 1986
283 mg/kg/d	5 mo	M	SVT	Hirsch & Zauder, 1986
≈1.875 g	5-9 y	?	VEB	Saarnivaara *et al.*, 1988
≈1.875 g	5-9 y	?	VEB	Saarnivaara *et al.*, 1988
1 g/d[a]	8 y	M	ST	Dean *et al.*, 1991
1 g[b]	4 y	F	PVC, VB	Seger & Schwartz, 1994

[a] Chloral hydrate was coadministered with furosemide.
[b] Chloral hydrate was administered 7.5 h after an accidental caffeine ingestion.

PVC: Premature ventricular contraction
ST: Sinus tachycardia
SVT: Supraventricular tachycardia
VB: Ventricular bigeminy
VEB: Ventricular ectopic beat

Table 3-2. Chloral hydrate-associated cardiotoxicity reported in adult subjects (adapted from Mayers, 1992).

DOSE	AGE	SEX	CARDIAC SYMPTOMS	REFERENCE
18 g	66 y	F	VT, VF	Gleich *et al.* , 1967
18 g	48 y	M	SVT, PVC, VT, VF	DiGiovanni, 1969
≈30 g	39 y	F	PAC, PVC	Gustafson *et al.* , 1977
≈25 g	51 y	M	VT	Gustafson *et al.* , 1977
≈20 g	21 y	F	VT	Gustafson *et al.* , 1977
15 g	29 y	F	VT	Marshall, 1977
10-20 g	62 y	F	VT	Marshall, 1977
17.5 g	19 y	F	ST, PVC	Vaziri *et al.* , 1977
38 g	38 y	F	PVC	Stalker *et al* ., 1978
14 g	17 y	M	VT	Bowyer & Glasser, 1980
30 g	67 y	F	VT	Bowyer & Glasser, 1980
10 g	29 y	F	ST, VT, VEB	Brown & Cade, 1980
37.5 g	36	F	VT	Brown & Cade, 1980
22.5 g	57	F	VT	Brown & Cade, 1980
15 g	30 y	F	PVC, SVT, torsade de pointes	Young *et al.* , 1986
?	42 y	M	VF, VT	Graham *et al.* , 1988
?	25 y	F	ST, VEB, VT	Graham *et al.* , 1988
≈15 g	26 y	F	ST, VEB, VT	Graham *et al.* , 1988
?	25 y	F	ST, VEB, VT	Graham *et al.* , 1988
?	20 y	F	ST, VEB	Graham *et al.* , 1988
?	46 y	F	ST, VEB, VT	Graham *et al.* , 1988
?	27 y	F	ST, VEB, VT	Graham *et al.* , 1988
?	23 y	F	ST, VEB, VT	Graham *et al.* , 1988
?	45 y	F	sinus rhythm, single VEB	Graham *et al.* , 1988
?	28 y	F	ST, VT, torsade de pointes	Graham *et al.* , 1988
?	42 y	M	ST, VEB	Graham *et al.* , 1988
≈8 g	42 y	F	PVC, VB	Jonville *et al.* , 1991
70 g	29 y	M	VT, VF	Ludwigs *et al.* , 1996

PAC: Premature atrial contraction
PVC: Premature ventricular contraction
ST: Sinus tachycardia
SVT: Supraventricular tachycardia
VB: Ventricular bigeminy
VEB: Ventricular ectopic beat
VF: Ventricular fibrillation
VT: Ventricular tachycardia

It has been postulated that the arrhythmogenic effect of chloral hydrate may be related to its structure and metabolism (Bowyer and Glasser, 1980; Brown and Cade, 1980). Chloral hydrate, trichloroethanol and trichloroacetic acid are all halogenated hydrocarbons, a number of which are known to have cardiotoxic effects (Figure 3-1). The mechanism of halothane-induced arrhythmias has generally been assumed to be sensitization of the myocardium to the effects of endogenous catecholamines (Jastak and Pallasch, 1988; Johnston *et al.*, 1976; Katz and Epstein, 1968; Marshall and Longnecker, 1990; Steward, 1985). Since β-adrenergic blocking agents have been shown clinically to consistently and specifically reverse halothane-induced arrhythmias, as well as those produced by chloral hydrate ingestion in patients, chloral hydrate and its metabolites are presumed to act in a manner similar to halothane in the production of arrhythmias (Bowyer and Glasser, 1980; Brown and Cade, 1980; Byatt and Volans, 1984; DiGiovanni, 1969; Graham *et al.*, 1988; Gustafson *et al.*, 1977; Hirsch and Zauder, 1986; Katz and Epstein, 1968; Komai *et al.*, 1991; Marshall, 1977). It has recently been proposed, however, that reductive dehalogenation of halothane, resulting in the formation of radical and carbanion complexes, may cause lipid peroxidation and the subsequent cardiotoxic effects (Gibson and Skett, 1986; Kharasch *et al.*, 1995; Spracklin *et al.*, 1995). Recent investigations have established that chloral hydrate, trichloroethanol and trichloroacetic acid generate free radicals with subsequent lipid peroxidation in an *in vitro* hepatic microsomal system (Ni *et al.*, 1995; Ni *et al.*, 1994; Ni *et al.*, 1996). The reductive dehalogenation of TCA with subsequent oxidative peroxyl radical formation and lipid peroxidation has also been proposed (Larson and Bull, 1992a). It is possible, then, that chloral hydrate-related arrhythmias result from free-radical mediated lipid peroxidation in the heart. Alternatively, chloral hydrate and/or one of its metabolites may act directly to enhance automaticity of supraventricular and ventricular pacemaker cells (Gustafson *et al.*, 1977).

There have been few scientific investigations of the potential of chloral hydrate to produce cardiac toxicity. An early study of the effects of chloral hydrate on the hearts of both dogs and horses proposed a primarily vagal mechanism in the development of arrhythmias (Gross and Hoff, 1968). These investigators proposed that the etiology of chloral hydrate-associated arrhythmias is related to secondary metabolic derangement which contributes to the cardiac toxicity (Gross and Hoff, 1968). Other investigators examined the arrhythmogenicity of trichloroethylene and its metabolites (trichloroethanol and trichloroacetic acid). They concluded that trichloroethylene, rather than its metabolites, sensitized the myocardium to epinephrine-induced arrhythmias (White and Carlson, 1981). No significant deterioration of a rat heart preparation was found when chloral hydrate (300 mg/kg) was used as the anesthetic agent prior to excision of the hearts (Riggs *et al.*, 1986). Another study of the anesthetic effects of chloral hydrate (300 and 400 mg/kg) in intact rats demonstrated that it severely depressed both the cardiovascular and respiratory systems (Field *et al.*, 1993). Recently, histochemical and enzymatic alterations in the heart following acute chloral hydrate administration were examined. The results of this investigation indicate that non-toxic doses of chloral hydrate can impair the metabolism of coronary vascular smooth muscle by attacking the rate-limiting enzymes of both aerobic and anaerobic metabolism in the coronary vasculature. Unfortunately, no attempt was made to determine chloral hydrate-metabolite concentrations (Trulson and Ulissey, 1987).

3.1.3.6 Subacute and Chronic Toxicity

3.1.3.6.1 Neurological Toxicity

A novel hypothesis implicating chloral hydrate in the development of neurodegenerative diseases such as Parkinson's disease has been recently described. A putative β-carboline neurotoxin (TaClo) is a structural analog of the known neurotoxin MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine)

and is believed to be derived from the spontaneous reaction of tryptamine (Ta) and chloral (Clo) *in vivo*. TaClo has recently been shown to form after administration of its precursor substances to rats. The neurotoxic potential of this compound on the dopaminergic system has been substantiated, and includes such effects as altered nigrostriatal dopamine metabolism, behavioral modification, and a decline in the population of dopaminergic neurons (Bringmann *et al.*, 1995). This finding has tremendous toxicological implications given the rather ubiquitous nature of chloral hydrate as both a therapeutic agent as well as an environmental contaminant.

3.1.3.6.2 Reproductive and Developmental Toxicity

A number of drinking water by-products have recently been implicated in a variety of adverse reproductive outcomes, such as inability to conceive, spontaneous abortion, and low birth weight (Klinefelter *et al.*, 1995). However, few of the specific contaminants have undergone reproductive toxicity testing. Dichloroacetic acid, a minor metabolite of both chloral hydrate and trichloroacetic acid (Larson and Bull, 1992a), has been shown to significantly affect epididymal sperm motility after chronic exposure (70 days) (Toth *et al.*, 1992). Dichloroacetic acid also appears to alter spermatogenesis (Toth *et al.*, 1992). A recent chronic (52-week) reproductive toxicity study demonstrated that daily exposure to 188 mg/kg chloral hydrate in F344 rats significantly reduced both the percentage of motile and progressively motile sperm. The fact that the adverse reproductive endpoints were observed prior to evidence of tumor development indicates that these endpoints may be more sensitive than tumorigenicity in the risk assessment of chloral hydrate and other drinking water byproducts (Klinefelter *et al.*, 1995).

The embryotoxicity of chloral hydrate, trichloroacetic acid and dichloroacetic acid has recently been assessed using a rat embryo model (Saillenfait, 1995). All three compounds produced concentration-dependent reductions in growth and differentiation and increases in the incidence of morphological abnormalities. Trichloroacetic acid and dichloroacetic acid

were roughly equipotent in this study, while chloral hydrate was slightly more potent (Saillenfait, 1995). Both trichloroacetic acid and dichloroacetic acid have been found to be teratogenic after oral administration in the rat (Saillenfait, 1995). A previous report indicated that there is an apparent increase in congenital cardiac malformations in children of mothers who live in areas with significant levels of halogenated hydrocarbon contamination of water supplies (Goldberg *et al.*, 1990). Substantiation of many of these developmental effects is lacking in humans. However, when used in the chronic management of convulsions in neonatal patients, chloral hydrate was found to restrict newborn weight gain (Moore, 1984).

3.1.3.6.3 Carcinogenicity

Chloral hydrate has been shown to be mutagenic in *Salmonella* tester strain TA100, yeast, fungi and *Drosophila* (Daniel *et al.*, 1992a). Aneuploidy has been reported following very high doses of chloral hydrate, leading to concerns of a carcinogenic potential in humans (American Academy of Pediatrics, 1993; Steinberg, 1993). Aneuploidy and sister chromatid exchanges have also been observed in human lymphocytes, cultured hamster ovary cells, and in mouse spermatogonia (Daniel *et al.*, 1992a; Fahrig *et al.*, 1995). Chloral hydrate exhibits a colchicine-like effect in rat hepatic microsomes, reversibly arresting cells in mitosis in a dose-dependent fashion (Steinberg, 1993). Oral administration of chloral hydrate has been shown to produce an increased incidence of single-strand hepatic DNA breaks in male B6C3F1 mice and Sprague-Dawley rats (American Academy of Pediatrics, 1993). However, the genotoxic potential of chloral hydrate is believed by some investigators to be minimal because it fails to induce cross-linking of DNA or proteins even at very high doses (Steinberg, 1993).

The principal target for chloral hydrate toxicity appears to be the liver, with large doses producing hepatic disease in all animal species studied (Daniel *et al.*, 1992a; Daniel *et al.*, 1992b; Sanders *et al.*, 1982; Steinberg, 1993). Hepatocellular necrosis and hepatocellular carcinomas induced

following very high chronic (2 year) doses of chloral hydrate in male (C57BL/6 X C3H)F_1 mice were similar to those observed after chronic trichloroethylene exposure (Daniel *et al.*, 1992a). Since two common metabolites of trichloroethylene and chloral hydrate (trichloroacetic acid and dichloroacetic acid) have been shown to cause liver tumors in the male B6C3F1 mouse, it is believed that these metabolic products may be responsible for chloral hydrate-induced hepatic tumorigenicity (Daniel *et al.*, 1992a; Daniel *et al.*, 1992b; Herren-Freund *et al.*, 1987; Larson and Bull, 1992b).

Several theories to explain the mechanism of the hepatotoxicity of chloral hydrate and its metabolites have been proposed. It has been suggested that the hepatocarcinogenicity of chloral hydrate may be related to the ability of trichloroacetic acid to inhibit gap junction-mediated intercellular communication. The gap junctions in plasma membranes permit the intercellular diffusion of low molecular weight molecules and ions, and appear to be required for regulation of cellular functions, including cell growth (Klaunig *et al.*, 1989). Metabolic activation of chloral hydrate by either reductive dehalogenation or CYP2E1-mediated oxidation to free radical intermediates appears to be an important determinant of hepatic toxicity, including tumorigenicity (Larson and Bull, 1992a; Ni *et al.*, 1996). The involvement of radical-induced lipid peroxidation in the etiology of the hepatotoxic effects of chloral hydrate and its metabolites has been well documented (Larson and Bull, 1992a; Ni *et al.*, 1995; Ni *et al.*, 1994; Ni *et al.*, 1996). Induction of hepatic peroxisomes by trichloroacetic acid and/or dichloroacetic acid radical species may also be a determining factor in the development of hepatocarcinogenesis. The oxidative stress produced by peroxisome proliferation would be expected to contribute to free radical-mediated cellular damage (Larson and Bull, 1992a; Larson and Bull, 1992b; Prout *et al.*, 1985).

The dose-response relationship for the carcinogenicity of chloral hydrate and its metabolites has been shown to be nonlinear in rodents. Chronic administration of disproportionately large doses of these agents are

required before the induction of malignancies is demonstrated (Daniel *et al.*, 1992a; Daniel *et al.*, 1992b; Steinberg, 1993). Additionally, hepatocarcinogenicity in female rodents and most strains of rat has not been documented (Daniel *et al.*, 1992a; Daniel *et al.*, 1992b; Sanders *et al.*, 1982). This gender/species difference may be related to a differential rate and/or extent of metabolism to trichloroacetic acid in susceptible mammals (Daniel *et al.*, 1992a; Daniel *et al.*, 1992b; Klaunig *et al.*, 1989; Larson and Bull, 1992a; Larson and Bull, 1992b; Prout *et al.*, 1985).

Trichloroethylene was banned from use in foods, drugs and cosmetic cleaning fluids after the discovery that very high chronic exposure induced malignancies in rodents (Steinberg, 1993). The recent demonstration of the tumorigenic and genotoxic effects of chloral hydrate and/or its metabolites in rodents has now resulted in efforts to restrict or ban the use of chloral hydrate as a therapeutic agent. However, the risk assessment for chloral hydrate carcinogenicity is based solely on *in vivo* studies carried out in rodents, and concern has been raised that linear extrapolation from chronic high dose exposure studies in rodents to humans may not be appropriate (American Academy of Pediatrics, 1993; Steinberg, 1993; Steinberg and DeSesso, 1993). Instead, it has been suggested that a threshold model for the medicinal use of chloral hydrate would be more relevant (Steinberg, 1993).

Consideration of the risks and benefits of a particular therapeutic agent should always be part of the medical decision-making process. Possible modifications in the use of chloral hydrate to achieve an acceptably low level of risk would include: avoiding prolonged sedation in neonates to prevent hyperbilirubinemia, excessive sedation and acidosis; restricting the use of chronic high doses of chloral hydrate in the pediatric population; limiting the use of chloral hydrate in patients with hepatocellular disease; and selecting alternative sedative agents when appropriate (American Academy of Pediatrics, 1993; Steinberg, 1993).

3.2 FREE RADICAL-MEDIATED TOXICITY IN THE HEART

3.2.1 Introduction

3.2.1.1 General Aspects of Free Radical Chemistry

A free radical is defined as "any species capable of independent existence that contains one or more unpaired electrons" (Halliwell, 1994). The simplest free radical, for example, is an atom of the element hydrogen, which contains one proton and a single electron. 'Reactive oxygen species' (ROS) is a general term that is widely used to refer to free radicals as well as molecular species that do not contain unpaired electrons, but which are instrumental in the generation of free radical species (Darley-Usmar and Halliwell, 1996; Halliwell, 1994). The generation of potentially deleterious oxygen free radicals is a natural consequence of aerobic life (Dargel, 1992; Halliwell, 1994). The formation of biologically reactive oxygen species (Figure 3-5) occurs as molecular oxygen is reduced by a series of electron transfer steps (Dargel, 1992; Loesser *et al.*, 1991; Yu, 1994). This process is believed to account for approximately 5% of the total cellular oxygen consumption (Rubanyi, 1988). Several reactive oxygen species are produced, each possessing a different degree of chemical reactivity (Dargel, 1992; Halliwell, 1994; Yu, 1994).

$$2\,O_2 + e^- \longrightarrow 2\,O_2^{\bullet-}$$
$$O_2^{\bullet-} + H_2O \longrightarrow HO_2^{\bullet} + OH^-$$
$$2\,O_2^{\bullet-} + 2\,e^- + 2\,H^+ \longrightarrow H_2O_2$$
$$H_2O_2 + e^- + H^+ \longrightarrow 2\,{}^{\bullet}OH$$
$${}^{\bullet}OH + e^- + H^+ \longrightarrow H_2O$$

Figure 3-5. Formation of reactive oxygen species during the univalent reduction of molecular oxygen, where e^-=electron; H^+=proton; O_2=molecular oxygen; $O_2^{\bullet-}$=superoxide anion; $HO_2^{\bullet}$= hydroperoxyl radical; OH^-=hydroxyl anion; H_2O_2=hydrogen peroxide; ${}^{\bullet}OH$=hydroxyl radical; H_2O=water (adapted from Loesser, 1991; Yu, 1994).

The first intermediate in the sequential univalent reduction of oxygen is the superoxide anion. A unique aspect of the superoxide anion is that while it reacts selectively with cellular targets, it can also lead to the formation of a variety of other ROS including the hydroxyl radical, hydrogen peroxide, singlet oxygen and the hydroperoxyl radical (Yu, 1994). Superoxide anion is produced for physiological purposes in phagocytic cells, where it assists in the inactivation of foreign microorganisms, and possibly in lymphocytes and fibroblasts, where it may be involved in intercellular signalling and growth regulation (Halliwell, 1994; Yu, 1994).

The hydroxyl radical is a highly reactive free radical which attacks almost all molecules in a diffusion-controlled fashion. Because of its reactive nature, the hydroxyl radical has a half-life measured in microseconds and generally damages molecules immediately adjacent to its site of formation (Halliwell, 1989; Halliwell, 1994; Yu, 1994). Hydroxyl radicals are formed primarily from the following reactions *in vivo* (Rubanyi, 1988; Yu, 1994):

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + {}^{\bullet}OH + OH^{-} \qquad \text{(Fenton reaction)}$$

$$O_2^{\bullet-} + H_2O_2 \longrightarrow O_2 + H_2O + {}^{\bullet}OH \qquad \text{(Haber-Weiss reaction)}$$

The toxicological significance of this radical is related to its ability to propagate free radical chain reactions (Halliwell, 1989).

Hydrogen peroxide can be formed by either the spontaneous or enzymatic dismutation of two superoxide anions (Yu, 1994). Hydrogen peroxide is a non-radical ROS which, because of its nonionized state, is very diffusable within and between cells (Halliwell, 1994; Rubanyi, 1988; Yu, 1994). This diffusability, coupled with the potential to generate the highly reactive hydroxyl radical, make hydrogen peroxide a very important cellular oxidant (Yu, 1994).

Pathological cellular production of free radicals can originate from either the impact of radiation (ionizing, ultraviolet, visible or thermal) or various electron transfer processes (catalyzed by transition metals or enzymes)

(Dargel, 1992). Certain pathophysiologic conditions can shift the normal metabolism of oxygen to univalent reductions with production of ROS (Loesser *et al.*, 1991). Specific sources of biologically important ROS include:

1. Xanthine/Xanthine Oxidase

   • Xanthine oxidase is a covalently modified form of the enzyme xanthine dehydrogenase which catalyzes the univalent oxidation of purine substrates with the concomitant production of the superoxide anion and possibly singlet oxygen (Loesser *et al.*, 1991; Prasad *et al.*, 1992a; Prasad *et al.*, 1989; Rubanyi, 1988; Willson, 1989; Yu, 1994).

   • Xanthine oxidase has been implicated in the pathophysiology of the ischemia/reperfusion phenomenon (Loesser *et al.*, 1991; Yu, 1994).

2. Stimulated Neutrophils

   • NADPH oxidase contained in the plasma membrane of activated neutrophils reduces oxygen to superoxide anion (Dargel, 1992; Loesser *et al.*, 1991; Willson, 1989).

   • Hydrogen peroxide formed from the dismutation of superoxide anion combines with chloride ion in the presence of myeloperoxidase to form hypochlorous acid. This highly reactive acid interacts with nitrogenous compounds to generate monochloramines, which are stable ROS capable of diffusing across plasma membranes (Dargel, 1992; Loesser *et al.*, 1991).

3. Electron Transport Systems

   • A potential source of both superoxide anion and hydrogen peroxide is the electron transport chain in the mitochondria (Dargel, 1992; Halliwell, 1994; Loesser *et al.*, 1991; Rubanyi, 1988; Yu, 1994). While only a small percentage of the reducing equivalents are believed to be diverted from mitochondrial energy production, this amount may be significant in terms of cellular toxicity (Halliwell, 1994).

• The electron transport systems of cytochromes P450 and b_5, contained in the microsomal and nuclear membranes, are also potential sources of superoxide anion and hydrogen peroxide (Dargel, 1992; Rubanyi, 1988; Willson, 1989; Yu, 1994).

4. Auto-oxidation Reactions

• Pathological production of superoxide anion results from auto-oxidation reactions in which certain compounds (i.e. catecholamines, tetrahydrofolate, reduced flavins) react directly with molecular oxygen to form the superoxide anion (Halliwell, 1994; Loesser *et al.*, 1991; Singal *et al.*, 1982).

5. Metabolism of Arachidonic Acid

• One of the enzymatic activities of prostaglandin H_2 synthase is as a hydroperoxidase which is responsible for the conversion of arachidonic acid to prostaglandin H_2. This process has been shown to generate a number of free radical species, including superoxide anion and singlet oxygen (Dargel, 1992; Loesser *et al.*, 1991; Rubanyi, 1988).

6. Calcium Overload

• It has been suggested that influx of calcium into the cell initiates free radical production (Loesser *et al.*, 1991). Calcium overload is known to damage cardiac mitochondria which may result in the diversion of molecular oxygen to univalent reduction pathways with the subsequent production of ROS (Loesser *et al.*, 1991).

• The calcium paradox is a phenomenon whereby reperfusion of previously ischemic myocardium with calcium immediately causes severe tissue damage. This phenomenon has been associated with the liberation of arachidonic acid from the plasma membrane, with subsequent production of free radicals (Loesser *et al.*, 1991).

Numerous cellular events have been identified as being consequences of ROS *in vivo.* Oxygen free radicals are chemically reactive species which can attack a variety of subcellular structures causing altered cellular metabolism and structure, and ultimately leading to cell death and necrosis (Loesser *et al.*, 1991). The categories of free radical mediated cellular damage include: covalent modification of biological structures like proteins or nucleic acids, which in many cases can only be corrected by resynthesis of the affected component; alterations of membrane structure and function as a result of peroxidation of the polyunsaturated fatty acid components of the plasma membrane; lipid peroxidation-associated generation of toxic non-radical products which have specific intracellular targets (Dargel, 1992; Halliwell, 1994; Loesser *et al.*, 1991; Yu, 1994).

3.2.1.2 Free Radicals and Oxidative Injury

It has become evident that protein molecules are highly sensitive to oxidative modification (Yu, 1994). The physical changes associated with oxidation of amino acid residues of proteins include fragmentation, aggregation and increased susceptibility to proteolytic digestion (Bolli, 1991; Dargel, 1992; Yu, 1994). Aggregation occurs by oxidative denaturation of the protein molecule, and may be related to the ability of the hydroxyl radical to form cross-linkages (Yu, 1994). Denatured proteins are more susceptible to degradation by proteolytic enzymes due to gross conformational changes (Halliwell, 1994; Yu, 1994). The interaction of free radicals with proteins and/or amino acids has serious physiological consequences. Covalent binding of free radicals to enzymes and receptors would be expected to alter cellular homeostatic mechanisms, thus contributing to cellular dysfunction and eventually cell death (Bolli, 1991; Dargel, 1992; Halliwell, 1989).

Nucleic acids are also very susceptible to oxidative attack by free radicals and ROS. Alteration of DNA molecules has obvious implications in the development of neoplastic transformations (Dargel, 1992). Oxygen free radicals may act as both direct and indirect carcinogens. Direct oxidative

damage of DNA induced by the hydroxyl radical includes both base alterations and strand breaks, with thymine and cytosine residues being most susceptible to oxidative attack (Yu, 1994). Indirect mechanisms of tumor induction include ROS-associated lipid peroxidation with subsequent production of potentially carcinogenic compounds (i.e. malondialdehyde), and depletion of intracellular antioxidant compounds (Yu, 1994).

Free radical-mediated membrane damage is perhaps the most important feature of oxidative injury. Peroxidative attack of the polyunsaturated fatty acid (PUFA) side-chains of membrane lipids involves the processes of initiation, propagation and termination (Dargel, 1992; Halliwell, 1989; Halliwell, 1994; Yu, 1994). Initiation of lipid peroxidation occurs following the attack of any species (i.e. a free radical) which is capable of abstracting a hydrogen atom from a PUFA side chain, with the coincident production of a carbon-centred radical (Halliwell, 1994; Yu, 1994). Propagation of peroxidation depends on the reaction of carbon-centred radicals with molecular oxygen to form the peroxyl radical, which can then attack adjacent PUFA side-chains and propagate the chain reaction (Dargel, 1992; Halliwell, 1994; Yu, 1994). Termination of a peroxidative chain reaction generally depends on the intervention of various components of the cellular antioxidant defense system.

Membrane damage associated with free radical attack includes: covalent binding of the free radical to membrane-associated enzymes and/or receptors; covalent binding of the radical to various membrane components; disturbance of membrane transport processes; initiation of peroxidation of PUFA side-chains of membrane lipids (Slater, 1984). These processes lead to degradation of the lipid membrane, interaction of degradation products with intra- and extracellular targets and generation of ROS during the lipid peroxidation chain reaction (Dargel, 1992). The non-radical products of lipid peroxidation consist primarily of alkanes, alkanals, alkenals and hydroxyalkenals, including malondialdehyde and 4-hydroxynonenal (Dargel, 1992; Darley-Usmar and Halliwell, 1996; Nair *et al.*, 1986; Slater, 1984;

Ungemach, 1987). These aldeyhydes react with both the sulfhydryl groups of thiols (protein and nonprotein) and the amino groups of proteins (Dargel, 1992; Nair *et al.*, 1986; Slater, 1984). Microsomal glucose-6-phosphatase and cytochrome P450 appear to be preferential targets of these lipid degradation products (Dargel, 1992). Alkenals also react with DNA, inhibit DNA repair, inhibit protein and RNA synthesis, and bind to glutathione (Dargel, 1992).

Free radical-mediated oxidative damage has been implicated in numerous clinical disorders, and is believed to be causally related to the development of atherosclerosis, rheumatoid arthritis, some forms of adult respiratory distress syndrome, the familial dominant form of amyotrophic lateral sclerosis, reoxygenation injury and traumatic or ischemic damage to the central nervous system (Halliwell, 1989; Halliwell, 1994). The susceptibility of a particular organ or tissue to the damaging effects of lipid peroxidation will vary depending on a number of factors, including: the architecture of the organ or tissue; the complex cellular composition; and the structural and functional properties of individual cell types (Dargel, 1992; Halliwell, 1994). Several disorders associated specifically with lipid peroxidation are: inflammation; carcinogenesis; atherogenesis; toxic cell injury; retinopathy of prematurity; ischemia and reperfusion of organs; myocardial injury (Dargel, 1992; Darley-Usmar and Halliwell, 1996; Halliwell, 1994). However, the causal relationship of lipid peroxidation in the pathogenesis of various diseases has not been rigorously established.

3.2.2 Myocardial Effects of Oxygen Free Radicals

It has been proposed that oxidative damage represents a fundamental mechanism of myocardial injury (Loesser *et al.*, 1991). Peroxidation of both proteins and lipids could be important factors in the etiology of cardiac toxicity (Bolli, 1991; Yu, 1994). Since myocytes have a limited ability to enzymatically detoxify ROS, the heart is particularly sensitive to ROS-induced damage (Milei *et al.*, 1986). Free radical-induced cardiomyopathy is associated with the development of ultrastructural damage, abnormal metabolism, transient

contractile failure (or myocardial stunning), arrhythmias and cell death (Singal *et al.*, 1982; Thollon *et al.*, 1995). The ischemia/reperfusion phenomenon is the most thoroughly investigated free radical-mediated myocardial pathology. Reperfusion injury refers to a variety of pathophysiologic derangements which manifest as cellular damage and dysfunction following the post-occlusion re-establishment of coronary blood flow (Bolli, 1991; Hudson, 1994). Other radical-induced cardiac pathologies share a number of features with the ischemia/reperfusion phenomenon, including the development of myocardial stunning, arrhythmias and cell death (Bolli, 1991; Jeroudi *et al.*, 1994). The ischemia/reperfusion related cardiotoxic effects can be used to illustrate the general pathogenesis of other radical-induced mechanisms of myocardial toxicity.

Ultrastructural damage which has been attributed to free radical-induced lipid peroxidation includes: early signs of fibrosis; vacuolization and granulation; cellular and mitochondrial swelling; loss of myofibrils; cellular necrosis; and infiltration of necrotic areas by polymorphonuclear leukocytes (Hearse *et al.*, 1989; Hudson, 1994; Singal *et al.*, 1982). The morphologic disturbances associated with adriamycin-induced lipid peroxidation consist of vacuolization of myocardial fibers which leads to severe myocytolysis (Milei *et al.*, 1986).

Intracellular calcium overload, which appears to be a consequence of peroxidation of the plasma and/or sarcoplasmic reticulum membranes, causes depletion of high-energy phosphates due to the activation of calcium-dependent ATPases and impairment of mitochondrial energy production (Bolli, 1991; Hudson, 1994; Singal *et al.*, 1982). Additionally, pathological processes which produce tissue hypoxia or anoxia promote anaerobic metabolism, leading to the accumulation of toxic metabolic by-products (Hudson, 1994). Anaerobic metabolism also depletes cellular stores of glycogen and ATP, with a concomitant inhibition of ATP-dependent membrane ion pumps (Hudson, 1994).

Myocardial stunning is a radical-mediated manifestation of myocardial injury (Bolli, 1991; Loesser *et al.*, 1991; Prasad *et al.*, 1992a; Prasad *et al.*, 1989). This mechanical dysfunction occurs in the absence of irreversible damage, and persists after reperfusion of previously ischemic tissue (Bolli, 1994; Hudson, 1994; Jeroudi *et al.*, 1994; Loesser *et al.*, 1991). Both the hydroxyl radical (Bolli, 1991; Jeroudi *et al.*, 1994) and singlet oxygen (Hearse *et al.*, 1989; Loesser *et al.*, 1991) have been implicated in the pathogenesis of myocardial stunning. There has been a consistent association between the prevention of ROS generation and attenuation of myocardial stunning (Bolli, 1991). Free radical-induced myocardial dysfunction may be initiated by peroxidation of sarcolemmal lipids, which then results in excitation-contraction uncoupling and intracellular calcium overload (Bolli, 1991; Jeroudi *et al.*, 1994; Loesser *et al.*, 1991; Prasad *et al.*, 1992a).

The extent of mechanical and electrophysiological disturbances following oxidative stress has been found to be correlated with the production of malondialdehyde (Nakaya *et al.*, 1987). The electromechanical alterations observed are also very suggestive of disturbed calcium homeostasis (Thollon *et al.*, 1995). It has thus been proposed that ventricular arrhythmias induced by free radical mechanisms may be initiated by a sudden burst of oxidative stress, which damages membrane lipids and proteins, leading to ionic imbalances and electrical instability (Cerbai *et al.*, 1991; Curtis *et al.*, 1993; Hearse *et al.*, 1989; Jeroudi *et al.*, 1994; Loesser *et al.*, 1991). Myocyte intracellular calcium overload produces oscillations in transmembrane potential (or afterdepolarizations), reductions in the arrhythmogenic thresholds and abnormal automaticity, all of which predispose the myocardium to various ventricular arrhythmias (Hudson, 1994; Jeroudi *et al.*, 1994; Nakaya *et al.*, 1987; Thollon *et al.*, 1995). These processes are all attenuated by antioxidant pretreatment (Cerbai *et al.*, 1991; Jeroudi *et al.*, 1994; Nakaya *et al.*, 1987). The available evidence suggests that there may be multiple types of cardiac injury caused by ROS, which possibly depend on the amount and/or type of ROS generated (Cerbai *et al.*, 1991).

Activated neutrophils have been implicated as mediators of tissue injury resulting in myocardial necrosis (Bolli, 1991). It has been postulated that massive infiltration of neutrophils in previously ischemic myocardium persists for extended periods of time, and could provide an ongoing source of oxygen free radicals (Bolli, 1991). Other investigators have suggested that peroxidative damage of the mitochondrial membrane results in disruption of the mitochondrial electrochemical gradient, with subsequent inhibition of ATP resynthesis. This process is believed to be integral in the development of irreversible cell injury during oxidative stress (Carini *et al.*, 1992). However, the role of ROS in the development of irreversible myocardial injury and necrosis is controversial (Bolli, 1991).

3.2.3 Cellular Defenses Against Oxidative Injury

Rising atmospheric oxygen tension caused a shift from primarily anaerobic life to the evolution of aerobic organisms which developed antioxidant defense systems to protect against the toxic effects of oxygen (Dargel, 1992; Halliwell, 1994; Parke *et al.*, 1990). These defense systems have evolved to provide adequate protection against 21% atmospheric oxygen, but are ineffective at higher oxygen tensions (Halliwell, 1994). Because the antioxidant defense systems are not completely effective, they work in concert with various repair systems which repair molecules that have sustained oxidative damage (Halliwell, 1994). Maximum protection against oxidative stress is provided by a multiplicity of both enzymatic and nonenzymatic systems capable of scavenging a variety of different ROS (Yu, 1994).

3.2.3.1 Enzymatic Antioxidants

3.2.3.1.1 Superoxide Dismutase

Superoxide dismutase (SOD) is an ubiquitous family of mammalian enzymes which catalyze the inactivation of excess superoxide anion with the subsequent generation of hydrogen peroxide and oxygen (Darley-Usmar and

Halliwell, 1996; Halliwell, 1994; Loesser *et al.*, 1991; Yu, 1994). This enzyme-catalyzed reaction proceeds approximately 10,000 times faster than the nonenzymatic chemical dismutation (Halliwell, 1994; Loesser *et al.*, 1991; Yu, 1994).

There are three classes of SOD enzymes, each with a distinct metal ion content. Mitochondrial SOD contains an essential manganese ion at its active site (MnSOD), while cyotsolic SOD has copper and zinc ions (Cu/ZnSOD) at its active site (Halliwell, 1994; Yu, 1994). An iron-containing SOD (FeSOD) is found primarily in the plasma (Yu, 1994).

SOD activity varies among various tissues, with the highest levels found in the liver, adrenal gland, kidney and spleen (Yu, 1994). Induction of the biosynthesis of SOD is regulated by tissue oxygenation and the presence of oxidant compounds (Yu, 1994).

Due to the generation of hydrogen peroxide, SOD enzymes work in collaboration with the hydrogen peroxide eliminating enzymes catalase and glutathione peroxidase (Halliwell, 1994; Yu, 1994).

3.2.3.1.2 Catalase

Catalase has an extensive tissue distribution, with particularly high activities demonstrated in the liver, kidney and erythrocytes (Yu, 1994). Catalase activity varies not only between tissues, but also within the cell (Yu, 1994). The highest intracellular activity of catalase is found in the peroxisomes (Halliwell, 1994; Yu, 1994).

The primary function of catalase enzymes is to convert hydrogen peroxide into water and oxygen; however, catalase is also responsible for the reduction of methyl and ethyl hydroperoxides (Halliwell, 1994; Loesser *et al.*, 1991; Yu, 1994). This biological function is shared with the glutathione peroxidase enzymes; however, catalase and glutathione peroxidase have different substrate specificities and affinities (Yu, 1994). Catalase is principally responsible for the metabolism of organic peroxides in the presence of high hydrogen peroxide concentrations (Yu, 1994).

3.2.3.1.3 Glutathione Peroxidase

The glutathione peroxidases (GSH-Px) are believed to be the most important family of hydrogen peroxide removing enzymes (Halliwell, 1994; Loesser *et al.*, 1991). Along with catalase, GSH-Px serves to catalyze the reduction of hydrogen peroxide, as well as various hydroperoxide species (lipid peroxides and lipoxygenase products), especially in the presence of low hydrogen peroxide levels (Yu, 1994).

The selenium-dependent GSH-Px enzymes contain an essential selenocysteine residue at the enzyme's active site (Halliwell, 1994). These cytosolic isozymes have a low capacity for the reduction of hydrogen peroxide, and may be involved in the degradation of phospholipid hydroperoxides (Yu, 1994). Selenium-independent GSH-Px preferentially utilize organic hydroperoxides as substrates (Yu, 1994).

The enzymatic reduction of hydrogen peroxide to water catalyzed by GSH-Px involves the concomitant oxidation of two molecules of glutathione. Oxidized glutathione is subsequently reduced by the enzyme glutathione reductase (Halliwell, 1994). Glutathione reductase is a flavoprotein which utilizes NADPH as a reducing equivalent in the regeneration of glutathione (Halliwell, 1994).

3.2.3.2 Non-enzymatic Antioxidants

3.2.3.2.1 α-Tocopherol

The generic term 'vitamin E' encompasses at least eight structural isomers of tocopherol, of which α-tocopherol exhibits the most potent antioxidant activity (Halliwell, 1989; Halliwell, 1994; Yu, 1994). Vitamin E is a lipophilic vitamin that is ubiquitously distributed in both the animal and plant kingdoms (Yu, 1994). The tissue distribution of α-tocopherol reflects its lipophilic nature, with the highest levels found in adrenal glands, heart, testes and liver (Yu, 1994).

The lipophilic nature of α-tocopherol allows it to distribute very widely in the body, especially within the lipid membranes of the mitochondria and endoplasmic reticulum, where it functions as the primary free radical chain terminator in the lipophilic environment (Dargel, 1992; Halliwell, 1989; Halliwell, 1994; Yu, 1994). α-Tocopherol is also responsible for the conversion of superoxide anion, hydroxyl radical and lipid peroxyl radicals to less reactive forms (Yu, 1994).

While controversy exists regarding the inherent status of α-tocopherol as either an antioxidant or pro-oxidant, it is generally believed that α-tocopherol provides a net antioxidant function in the cell (Dargel, 1992; Darley-Usmar and Halliwell, 1996). The controversy has arisen because α-tocopherol has some reducing properties, and also forms a short-lived radical species which is recycled *in vivo* by ascorbic acid, glutathione and/or ubiquinol (Dargel, 1992; Darley-Usmar and Halliwell, 1996; Halliwell, 1989; Halliwell, 1994).

3.2.3.2.2 β-Carotene

β-Carotene is a metabolic precursor of the fat-soluble vitamin A (Yu, 1994). Like α-tocopherol, β-carotene is located primarily within the lipophilic membranes of tissues (Yu, 1994).

Carotenoids, including β-carotene, are capable of directly quenching various ROS, notably singlet oxygen, superoxide anion and peroxyl radicals (Dargel, 1992; Milei *et al.*, 1986; Yu, 1994). The long chain of conjugated double bonds of β-carotene suggests its facility as a free radical scavenger (Yu, 1994). β-Carotene is particularly effective in the prevention of lipid peroxidation induced by xanthine oxidase (Yu, 1994).

Like α-tocopherol, β-carotene appears to possess both antioxidant and pro-oxidant activities (Yu, 1994). Pro-oxidant effects are demonstrated particularly at high oxygen partial pressures (Yu, 1994).

3.2.3.2.3 Ascorbic Acid

Ascorbic acid, or vitamin C, is a hydrophilic vitamin which has a wide distribution in intracellular and extracellular fluids (Halliwell, 1994; Yu, 1994). The ascorbate anion is the predominant form at physiological pH (Yu, 1994). Relatively high levels of ascorbate are found in the adrenal and pituitary glands, and lower concentrations are found in the liver, spleen, pancreas and brain (Yu, 1994).

Ascorbate is a reducing agent and antioxidant, which directly scavenges superoxide anion, singlet oxygen, hydroxyl radical and various lipid hydroperoxides (Dargel, 1992; Halliwell, 1994; Yu, 1994). Ascorbate is known to provide the most effective protection against plasma lipid peroxidation (Yu, 1994). As mentioned previously, one of the primary functions of ascorbate is to recycle the α-tocopherol radical (Darley-Usmar and Halliwell, 1996; Halliwell, 1994; Yu, 1994).

Excess amounts of ascorbic acid may have pro-oxidant activity in the presence of transition metals by generating superoxide anion, hydrogen peroxide and hydroxyl radical with subsequent induction of lipid peroxidation (Halliwell, 1994; Yu, 1994). It has been suggested that the concentration and the subcellular distribution are the major determinants of the pro-oxidant nature of ascorbate (Yu, 1994).

3.2.3.2.4 Glutathione

Glutathione (GSH) is a tripeptide, γ-glutamyl-cysteine-glycine, which is present in virtually all mammalian cells (Bray and Taylor, 1993; Dargel, 1992; Yu, 1994). The γ-glutamyl bond confers resistance to peptidase attack (Yu, 1994). GSH has a primarily intracellular distribution (concentrations 0.5-10

mM), although it is also located in the alveoli of the lung (Dargel, 1992; Yu, 1994). Under normal physiologic conditions less than 5% of cellular glutathione exists in its oxidized (GSSG) form, and constant regeneration of reduced GSH by glutathione reductase is essential for cellular integrity (Dargel, 1992).

The ability of GSH to react with both electrophiles and oxidants makes it an especially versatile component of the cellular detoxification system (Bray and Taylor, 1993; Yu, 1994). Depletion of intracellular GSH by various chemical interventions predisposes the cell to lipid peroxidation and cellular injury (Dargel, 1992). In addition to its role as a substrate for GSH-Px enzymes, reduced glutathione is capable of directly scavenging free radicals, particularly hydroxyl and carbon-centred radicals (Halliwell, 1994; Yu, 1994). Another important function of GSH is related to the detoxification of the degradation products of lipid peroxidation, a reaction catalyzed by the enzyme glutathione S-transferase (Darley-Usmar and Halliwell, 1996; Yu, 1994).

3.2.3.2.5 Other Antioxidant Compounds

The antioxidant activity of uric acid, an end product of purine metabolism in primates, has recently been recognized (Halliwell, 1989; Yu, 1994). At physiological pH, the anion form (urate) predominates. Urate has a wide physiological distribution, acting as a major extra- and intracellular component of the antioxidant defense system (Yu, 1994). Urate is capable of scavenging the hydroxyl radical, superoxide anion, oxoheme oxidants and peroxyl radicals (Yu, 1994). The ability of urate to complex transition metals may be involved in its sparing action on plasma ascorbate (Yu, 1994).

Glucose is an ubiquitous carbohydrate which is utilized as a primary energy source in mammalian systems. The antioxidant activity of glucose stems from its ability to scavenge the hydroxyl radical *in vivo* (Yu, 1994).

Cysteine is a widely distributed amino acid which is capable of reducing a variety of organic compounds by the donation of an electron from its sulfhydryl group (Yu, 1994).

Histidine may function as a scavenger of singlet oxygen *in vivo* (Hearse *et al.*, 1989).

Bilirubin, a product of hemoprotein catabolism, functions as a chain-breaking antioxidant by reacting with peroxyl radicals (Halliwell, 1989; Yu, 1994).

3.3 DEVELOPMENTAL ASPECTS OF MYOCARDIAL RESPONSE

3.3.1 Introduction

Investigations in neonatal animals strongly suggest that the neonatal heart undergoes physiological and morphological changes after birth which are both extensive and important (Brus and Jacobowitz, 1972; Case *et al.*, 1989; Chen *et al.*, 1979; Ezrin *et al.*, 1983; Ferrer, 1977; Friedman, 1972; Friedman *et al.*, 1968; Geis *et al.*, 1975; Nakanishi and Jarmakani, 1984; Olley and Rabinovitch, 1985; Pappano, 1977; Roffi and Motelica-Heino, 1975; Rosen and Hordof, 1977). This period of transition is characterized by enhanced sensitivity of the developing myocardium to numerous exogenous stimuli, including the administration of pharmacologic agents (Ferrer, 1977). Endogenous disturbances such as hypoxemia, acidosis and metabolic or ionic derangements can also contribute to the excessive lability of the heart in the immediate postnatal period (Ferrer, 1977).

Increased cardiac monitoring in neonatal patients has focused attention on the incidence of arrhythmias in this population. Contrary to previous beliefs, dysrhythmias in neonates, especially premature neonates, are relatively common (Case *et al.*, 1989; Church *et al.*, 1967; Grifka and Garson, 1991; Morgan *et al.*, 1965; Rogers and Richmond, 1978; Välimäki, 1969). Hypoxia, acidosis and electrolyte disturbances all contribute the development of arrhythmias in the neonatal population (Olley and Rabinovitch, 1985). It has been observed that virtually all newborn infants experience sinus tachycardia, which is believed to be the predominant rhythm in the immediate postnatal period (Church *et al.*, 1967; Morgan *et al.*, 1965; Morgan and Guntheroth,

1965; Rogers and Richmond, 1978). Sinus bradycardia with or without nodal escape is also quite common, especially in premature neonates (Church *et al.*, 1967; Morgan *et al.*, 1965; Morgan and Guntheroth, 1965). Autonomic immaturity is believed to contribute to the high incidence of ectopic beats (both atrial and ventricular) and supra-ventricular tachycardia in newborn infants (Morgan *et al.*, 1965; Morgan and Guntheroth, 1965; Rogers and Richmond, 1978). The most common neonatal arrhythmias have been shown to be premature atrial contractions, premature ventricular contractions, supraventricular tachycardia and atrial flutter (Grifka and Garson, 1991). The premature or asphyxiated infant is especially vulnerable to the development of more serious arrhythmias (Rogers and Richmond, 1978).

An understanding of the basis of the developmental differences observed in the neonatal myocardium is crucial for the rational implementation of therapeutic interventions. The developmental aspects of the myocardial response to therapeutic and toxicologic agents include immaturity of structural, biochemical and physiological components of the myocardium.

3.3.2 Anatomical Differences

Growth rates of various cardiac morphological parameters (mitral valve ring dimension, tricuspid valve ring dimension and total cardiac dimension) demonstrate that continuous myocardial growth occurs from the fetal stage to approximately 3 1/2 years of age (Ishii *et al.*, 1990). Myocardial growth is thought to significantly exceed development of the neonatal guinea pig coronary bed (Achterberg *et al.*, 1988). It has been further demonstrated that age-dependent alterations in the topology of the intercellular junctions responsible for electrical (gap junctions) and mechanical (fasciæ adherentes) coupling continue until about 6 years of age. These spatial modifications appear to parallel the changing ventricular functional requirements by accomodating an initial need for rapid growth-dependent remodeling, and gradually evolving to a relatively stable and rapidly conducting adult myocardium (Peters *et al.*, 1994; Spach, 1994).

Immature myocytes are smaller than adult myocytes, and contain a higher proportion of non-contractile (i.e. mitochondria, nuclei and surface membranes) to contractile (i.e. myofilaments) elements (Achterberg *et al.*, 1988; Friedman, 1972; Nakanishi and Jarmakani, 1984; Olley and Rabinovitch, 1985). The contractile elements of the adult heart comprise approximately 60% of the total muscle mass, compared with only 30% in the neonatal heart (Friedman, 1972). Reduced myofibrillar volume in neonatal myocytes is accompanied by a relative disorganization of the myofibrillar units and the extracellular matrix network. Age-dependent maturation of these mechanical and structural components in the developing myocyte contributes to increased mechanical efficiency of the myofibrils (Vannier *et al.*, 1996). The developing rabbit heart contains a slow skeletal muscle isoform of troponin I, in addition to the normal cardiac isoform, that is not found in adult myocytes. The slow skeletal troponin remains the predominant isoform throughout fetal life, being replaced by the cardiac isoform by about 9 months of postnatal development. This altered developmental troponin expression would be expected to confer different contractile properties on the immature myocardium (Sasse *et al.*, 1993; Vannier *et al.*, 1996).

A higher water and protein content (on a per gram basis) makes the structure of the immature myocardium relatively denser than that of the mature heart (Hammon, 1995). Neonatal myocytes are also comprised of an assembly of immature and nonhomogenous sarcomeres (Olley and Rabinovitch, 1985). These structural differences may account for the reduced compliance and lower preload reserve noted in neonatal hearts (Friedman, 1972; Hammon, 1995; Olley and Rabinovitch, 1985).

3.3.3 Biochemical Differences

3.3.3.1 Energy Catabolism

The endogenous glycogen stores in the immature myocardium are plentiful, leading to an increased use of glucose metabolism from

glycogenolysis for its energy requirements (Hammon, 1995; Lopaschuk *et al.*, 1992). Additionally, the neonatal heart demonstrates more effective anaerobic metabolism than does the adult heart (Achterberg *et al.*, 1988; Chiu and Bindon, 1987; Hammon, 1995; Lopaschuk *et al.*, 1992). The increase in glycolytic capacity in neonatal mycocardium may be related to increased activities of key glycolytic enzymes (Lopaschuk *et al.*, 1992).

The oxidative capacity of the developing rat ventricle (as estimated by the oxygen consumption or maximal respiration rate) is lower in immature rat ventricles than in mature ventricles (Lopaschuk *et al.*, 1992; Vannier *et al.*, 1996). Creatine does not stimulate respiration in immature rat ventricles, which implies a lack of coupling between oxidative phosphorylation and mitochondrial creatine kinase in immature tissue (Vannier *et al.*, 1996).

A relative deficiency of 5'-nucleotidase in the immature myocardium contributes to enhanced high-energy phosphate homeostasis. (Achterberg *et al.*, 1988; Grosso *et al.*, 1992; Hammon, 1995). 5'-Nucleotidase is responsible for the metabolic conversion of adenosine monophosphate (which is nondiffusible) to adenosine (which is freely diffusable). Maintenance of intracellular purine levels would contribute to the preservation of the high-energy phosphate pool (Grosso *et al.*, 1992).

The reduced activity of calcium adenosine triphosphatase in the sarcoplasmic reticulum results in lower intracellular calcium sequestration in immature hearts. Calcium-mediated excitation-contraction coupling in the neonatal myocardium is thus more dependent on extracellular calcium stores than in the adult heart (Hammon, 1995; Hatem *et al.*, 1995; Nakanishi and Jarmakani, 1984). Calcium sensitivity of myofibrillar force development is higher in newborn rat ventricles than in adult ventricles (Vannier *et al.*, 1996). This may be a result of developmental alterations of troponin I isoforms (Sasse *et al.*, 1993; Vannier *et al.*, 1996).

3.3.3.2 Endogenous Cellular Defenses

Aging has long been associated with increased susceptibility to oxidative attack (Cusack *et al.*, 1991; Scarpa *et al.*, 1987). Antioxidant defenses are known to diminish in aging mammals. It has been suggested that age-dependent reduction in the levels of reduced glutathione may be related to an age-related increase in the cellular oxidation rate, or to a reduction in the overall GSH turnover (increased degradation and/or reduced biosynthesis) (Cusack *et al.*, 1991; Yu, 1994). An age-related reduction in glutathione peroxidase activity would contribute to the decreased availability of glutathione (Cusack *et al.*, 1991). However, neonatal animals may actually be provided with enhanced antioxidant defenses as a preparation for the transition from a relatively hypoxic environment *in utero* to the oxygen-rich postnatal atmosphere (Frank and Groseclose, 1984; Scarpa *et al.*, 1987; Stevens and Autor, 1977). For example, the activities of superoxide dismutase, catalase and/or glutathione peroxidase exhibit a similar maturational increase in the final 3-5 days prior to parturition in the developing lung (Frank and Groseclose, 1984; Stevens and Autor, 1977), liver (Yang *et al.*, 1993) and brain (Juchau, 1990; Scarpa *et al.*, 1987).

A recent investigation of oxidative injury in neonatal rats has shown that the immature myocardium has multiple protective mechanisms, including: reduced oxidant production resulting from lower xanthine oxidase activity; enhanced ROS scavenging activity (specifically catalase activity); and increased expression of certain heat shock proteins (HSP72), which are associated with myocardial protection from a wide range of stressful stimuli (Rowland *et al.*, 1995).

The activity of xanthine oxidase has been reported to be roughly five times lower in neonatal *versus* adult rat hearts (Achterberg *et al.*, 1988; Rowland *et al.*, 1995). Both cardiac and hepatic xanthine oxidase exhibit linear postnatal increases in activity (Achterberg *et al.*, 1988; Schoutsen and de Jong, 1987). The potential benefits of reduced xanthine oxidase activity in neonatal hearts would include: higher hypoxanthine levels, which would

result in more efficient reincorporation of purine bases into the myocardial ATP pool; and reduced generation of ROS under conditions of myocardial ischemia and toxic insult (Achterberg *et al.*, 1988).

3.3.4 Physiological Differences

3.3.4.1 Autonomic Nervous System

3.3.4.1.1 Sympathetic Nervous System

Sympathetic innervation is either incomplete or functionally immature in the fetus and early newborn (Brus and Jacobowitz, 1972; Ezrin *et al.*, 1983; Ferrer, 1977; Friedman, 1972; Friedman *et al.*, 1968; Geis *et al.*, 1975; Gennser and Nilsson, 1970; Pappano, 1977; Partanen and Korkala, 1974; Roberts, 1991). Evidence from experimental animals suggests that norepinephrine stores are lower in the immature heart, and are located primarily in the preterminal nerve trunks rather than in the terminal nerve endings (Friedman, 1972; Friedman *et al.*, 1968; Gennser and von Studnitz, 1975; Mirkin, 1972; Olley and Rabinovitch, 1985; Papka, 1974). Adult levels of cardiac catecholamines are achieved by approximately the third postnatal week (Roberts, 1991). The adrenal glands at a comparable developmental stage contain significant catecholamine stores, suggesting that adrenal release of catecholamines may play a critical role in the maintenance of ventricular contractility in the immature myocardium (Friedman, 1972; Friedman *et al.*, 1968; Geis *et al.*, 1975; Roffi and Motelica-Heino, 1975).

While extrinsic sympathetic innervation occurs relatively late in the developmental process, considerable evidence suggests that not only are β1-adrenergic receptors fully functional early in pregnancy, but they also exist in dramatically greater numbers in the immediate postnatal period than in adulthood (Brus and Jacobowitz, 1972; Chen *et al.*, 1979; Geis *et al.*, 1975; Gennser and Nilsson, 1970; Olley and Rabinovitch, 1985; Pappano, 1977; Rane, 1980; Steinberg *et al.*, 1991). There is evidence, however, that the

coupling of β-adrenergic receptors to adenylate cyclase in neonatal myocardium is functionally incomplete (Artman *et al.*, 1988). The increase in neonatal receptor density parallels the marked increase in myocardial sensitivity to catecholamines, which has been referred to as "denervation hypersensitivity." The immature myocardium is more sensitive to both the positive chronotropic and positive inotropic effects of noradrenaline and adrenaline than is the adult myocardium (Ezrin *et al.*, 1983; Friedman, 1972; Geis *et al.*, 1975; Olley and Rabinovitch, 1985; Rogers and Richmond, 1978; Rosen and Hordof, 1977). The degree of adrenergic supersensitivity in immature hearts declines in the first week(s) after birth until adult responses are attained (Rogers and Richmond, 1978).

3.3.4.1.2 Parasympathetic Nervous System

Ontogenesis of the parasympathetic nervous system is qualitatively similar to the sympathetic nervous system, with the post-synaptic components (i.e. receptors) appearing prior to the presynaptic components (i.e. efferent nerves) (Pappano, 1977; Schwieler *et al.*, 1970). The parasympathetic nervous system, however, develops significantly faster than the sympathetic nervous system (Pappano, 1977).

The response of the neonatal myocardium appears to be similar to that of the adult myocardium, suggesting that the parasympathetic nervous system (PNS) is fully functional at birth (Friedman, 1972; Olley and Rabinovitch, 1985). Functional inhibition of fetal cholinergic receptors in the sino-atrial node has been demonstrated after atropine administration to pregnant women (Rane, 1980). However, the immature contractile response to exogenously administered cholinergic agents is lower than that of the adult myocardium (Coltart *et al.*, 1971). Additionally, certain investigators have shown that the resting vagal tone is lower in the neonatal *versus* the adult heart (Geis *et al.*, 1975).

Cholinergic innervation of the atrium, sino-atrial node, atrio-ventricular node and ventricles is comparable in immature and mature myocardium

(Friedman, 1972; Gardner and O'Rahilly, 1976). However, the functional response of the sino-atrial node to the inhibitory effects of acetylcholine increases with postnatal age until a maximal adult level is attained (Pappano, 1977). The enhanced sensitivity of the neonatal pacemaker to the negative chronotropic effects of acetylcholine have been attributed to lower levels of acetylcholinesterase, which would prolong the effects of cholinergic stimulation (Pappano, 1977).

3.3.4.2 Cellular Electrophysiology

There are developmental differences in cardiac action potentials that are reflected by postnatal changes in cellular electrophysiology and drug responsiveness (Case *et al.*, 1989; Ezrin *et al.*, 1983). The resting and action potential amplitudes from both atrial and ventricular myocytes are lower in immature hearts compared to adult hearts (Coltart *et al.*, 1971; Ezrin *et al.*, 1983; Rosen *et al.*, 1977). Developmental alterations in the shape, duration, and rate-dependence of the duration in neonatal atrial action potentials have been observed (Crumb *et al.*, 1995; Rosen *et al.*, 1977).

It has been proposed that action potentials from immature myocytes reflect an ongoing ionic maturational process (Ezrin *et al.*, 1983). There are significant differences in the cardiac excitation and repolarization processes in immature hearts. An increased density of sodium channels (associated with excitatory events) may be responsible for a steeper membrane-responsiveness curve in neonatal rabbit Purkinje fibers (Ezrin *et al.*, 1983). The transient outward potassium current in human neonatal atria and ventricles is much less dense than that found in adult ventricular myocytes. Substantial postnatal increases in the inwardly rectifying potassium current have also been demonstrated in both rat and rabbit ventricular myocytes. (Crumb *et al.*, 1995). These developmental differences in the potassium currents would be expected to significantly contribute to alterations in the repolarization processes in neonatal hearts.

The electrophysiologic processes regulating excitation-contraction coupling also undergo important maturational changes. It has been suggested that, due to immaturity of the L-type calcium channel, initiation of contraction is mediated largely by influx of calcium via the sodium/calcium exchanger (Hatem *et al.*, 1995). However, calcium-induced sarcoplasmic reticular calcium release has been documented as early as the third postnatal day (Hatem *et al.*, 1995).

3.3.4.3 Functional Responses

Investigations of the developmental aspects of myocardial mechanical function in fetal, neonatal and adult rabbit hearts have demonstrated that the immature myocardium develops less force than the adult heart (Artman *et al.*, 1988; Friedman, 1972). It has been suggested that this lesser force of contraction may be related to reduced intracellular calcium concentration, myofibrillar content and ATPase activity in the immature heart (Friedman, 1972; Nakanishi and Jarmakani, 1984). The dependency of the immature myocardium on extracellular calcium for excitation-contraction coupling is illustrated by the exaggerated depressant effect of calcium channel blockers in neonates (Hammon, 1995). Normal immature hearts also have a lower rate of maximum tension development and reduced inotropic reserve, and tend to operate under maximal adrenergic stimulation (Hammon, 1995; Nakanishi and Jarmakani, 1984; Olley and Rabinovitch, 1985). These factors contribute to the enhanced inotropic response of the immature heart to anesthetic agents and the unpredictable response to pressor agents (Hammon, 1995; Mirkin, 1972). The different response of developing myocardium to β-adrenergic stimulation may be at least partially attributed to the postnatal transition from a predominantly slow skeletal muscle troponin I isoform to the exclusive expression of the cardiac troponin I in older hearts (Sasse *et al.*, 1993). The extent of β-adrenergic blockade following administration of β-adrenergic blocking agents is similar in immature and adult hearts (Friedman, 1972).

It is generally believed that the immature myocardium is more tolerant to the effects of hypoxia and ischemia compared to the adult heart. Neonatal hearts demonstrate better recovery of contractile function, less creatine kinase release and lower left ventricular end diastolic pressure *versus* adult hearts (Rowland *et al.*, 1995). Tolerance of immature hearts to the effects of hypoxia and ischemia may be related to: increased glycogen stores; better anaerobic glycolytic capacity; improved maintenance of ischemic calcium exchange; higher concentrations of ATP substrates; lower activity of 5'-nucleotidase; and better amino acid substrate utilization (Grosso *et al.*, 1992; Hammon, 1995; Lopaschuk *et al.*, 1992; Rowland *et al.*, 1995). Recent studies, however, have suggested that myocardial lactate accumulation resulting from anaerobic metabolism during global ischemia may contribute to toxicity in neonatal patients. The neonatal myocardium would still be expected to be resistant to the deleterious effects of hypoxia, since the preservation of coronary flow would ensure adequate washout of excess lactate (Chiu and Bindon, 1987).

4. EFFECTS OF CHLORAL HYDRATE & ITS METABOLITES ON THE ISOLATED PERFUSED ADULT RABBIT HEART

4.1 INTRODUCTION

Chloral hydrate (CH) is the oldest sedative-hypnotic still in clinical use (Butler, 1970; Hoskins, 1984). While once employed extensively in almost all patient populations, CH administration is now limited almost exclusively to pediatric and geriatric patients (Cook *et al.*, 1992; Hoskins, 1984; Marx *et al.*, 1993). In addition to therapeutic administration, significant environmental exposure to CH and TCA can occur inadvertently due to drinking water contamination in chlorine disinfected water supplies (U.S. Environmental Protection Agency, 1975; U.S. Environmental Protection Agency, 1982; Uden and Miller, 1983).

Chloral hydrate is rapidly metabolized *in vivo* both by reduction to trichloroethanol and by oxidation to trichloroacetic acid. When administered to healthy adult volunteers, CH is nearly undetectable within minutes of administration (Breimer *et al.*, 1974; Gorecki *et al.*, 1990). TCE is glucuronidated prior to excretion in the urine (Breimer *et al.*, 1974; Sellers *et al.*, 1978). The elimination half-life of TCE is approximately 8-12 hours; that of the glucuronide conjugate is about 6-8 hours (Breimer *et al.*, 1974; Sellers *et al.*, 1978). TCA, a potentially toxic metabolite, has a half-life of 67-100 hours (Breimer *et al.*, 1974; Sellers *et al.*, 1978), leading to the concern that it may persist in the body (Hindmarsh *et al.*, 1991; Müller *et al.*, 1974; Sellers *et al.*, 1978).

A number of serious toxic reactions have been attributed to CH ingestion. In particular, CH has been plagued by anecdotal and case reports of cardiac arrhythmias, especially in situations of overdose (Graham *et al.*, 1988; Gustafson *et al.*, 1977; Hirsch and Zauder, 1986; Marshall, 1977). It has

been estimated that approximately 25% of CH intoxications will result in extrasystoles (Wiseman and Hampel, 1978). Few investigations of the direct effects of CH and/or its metabolites (TCE and TCA) on the heart have been reported (Gross and Hoff, 1968; Riggs *et al.*, 1986; Trulson and Ulissey, 1987; White and Carlson, 1981).

The present investigation used the isolated perfused adult rabbit heart as a model system to determine the cardiotoxic potential of CH and/or its metabolites, and to elucidate the levels of these compounds associated with cardiotoxicity.

4.2 EXPERIMENTAL

4.2.1 Materials

4.2.1.1 Chemicals

Chloral hydrate was obtained from Bristol-Myers Squibb Canada, Inc. (Montreal, PQ), trichloroacetic acid was purchased from Fluka Chemicals, Terochem Laboratories Ltd. (Edmonton, AB), and trichloroethanol was obtained from Aldrich Chemical Co. (Milwaukee, WI). All other chemicals were purchased from either Sigma Chemical Co. (St. Louis, MO) or BDH Chemicals (Toronto, ON). All chemicals used were of reagent grade.

4.2.1.2 Animals

New Zealand adult white rabbits (2-3 kg) of either sex were obtained from the licensed breeding facility at the Animal Resources Centre, University of Saskatchewan (see Appendix A, Table A-2). All animals were housed in stainless-steel cages with access to standard rabbit laboratory chow and tap water *ad libitum*, in accordance with the recommendations of the Canadian Council on Animal Care (CCAC).

4.2.2 Methods

4.2.2.1 Heart Perfusion

Animals were sacrificed by cervical dislocation according to accepted CCAC protocols. Anesthetic exposure was avoided to prevent any potential for interaction with the subsequent drug (CH, TCE, or TCA) treatments. The hearts were rapidly excised and immediately placed in ice-cold perfusion buffer. The aorta was cannulated as quickly as possible (within 1-2 minutes) and Langendorff perfusion (Langendorff, 1895) was initiated at a high flow rate to clean the coronary arteries. Once the arteries were sufficiently flushed, the heart was perfused as described previously (Prasad *et al.*, 1992b). Retrograde perfusion was maintained at a constant pressure of 100 cmH_2O with a bicarbonate buffer, pH 7.4, of the following composition (in mM): sodium chloride, 154.0; potassium chloride, 5.63; sodium bicarbonate, 5.95; calcium chloride, 2.16; and glucose, 5.55. The buffer passed through an in-line filter prior to heart perfusion, was equilibrated with a gas mixture of 95% O_2 and 5% CO_2 and was maintained at 37°C.

A small incision was made in the left atrium, and a balloon catheter was introduced into the left ventricle through the mitral valve. The balloon was filled with normal saline solution with a volume sufficient to raise the left ventricular end-diastolic pressure to 5 mmHg. The catheter was attached to a Gould pressure transducer (Gould Inc. Cardiovascular Products Division, Oxnard, CA) and a Beckman R411 dynograph recorder. Lead II ECG was monitored and recorded simultaneously. The heart was perfused at a rate of 11.6 ± 0.6 mL/minute, and was allowed to stabilize for at least 45 minutes prior to any interventions.

4.2.2.2 Myocardial Assessment

The mechanical parameters determined from the recordings were: left ventricular developed pressure (LVDP = LVSP - LVEDP; mmHg); dP/dT (mmHg/min; a reflection of myocardial contractility); and 4) index of myocardial

oxygen consumption (MVO_2 = LVSP x HR; mmHg/min). The first derivative of LVDP (dP/dT) was used to assess left ventricular contractility in the isolated perfused heart. These parameters were determined every 10 minutes throughout the protocol. Coronary flow (CF; mL/min) was determined by collecting the perfusate over one minute.

Lead II ECG was monitored for the assessment of cardiac arrhythmias. The diagnostic ECG parameters were: heart rate (beats/min); P-R interval (seconds; an indication of atrio-ventricular conduction); QRS duration (seconds; an indication of conduction within the ventricles); ectopic beats (atrial and ventricular); ventricular tachycardia; and ventricular fibrillation (which was considered to be the end point of arrhythmia). These parameters were recorded every 10 minutes throughout the experimental protocol, and more frequently if the situation warranted close monitoring.

4.2.2.3 Drug Administration

Drug solutions (CH 50, 100, 200 µg/ml or 0.30, 0.61, 1.2 mM; TCE 25, 50, 100, 150 µg/ml or 0.17, 0.34, 0.67, 1.0 mM; and TCA 25, 50, 100, 150 µg/ml or 0.15, 0.31, 0.61, 0.92 mM) were prepared fresh daily using the buffer as solvent. The perfusion apparatus was connected such that separate reservoirs for pure buffer and drug/buffer solutions could be employed. Drug administration via the buffer solution was initiated after stabilization of the heart and continued for 60 minutes (treatment phase). Following the treatment phase, the perfusion was switched back to pure buffer and the heart was monitored for an additional 60 minutes (recovery phase). Ten control experiments (with treatment and recovery phases analogous to treated hearts) were performed, while the drug interventions were repeated 6-7 times for each dosing level (see Appendix A, Table A-1).

4.2.2.4 Expression of Results

Individual experimental parameters were time-averaged over the course of the treatment and recovery phases (responses were calculated every 10 minutes during the protocol), by calculating the areas under the response *vs.* time curve (AUC's) using Lagrange interpolation (Rocci and Jusko, 1983). The AUC's for the treatment phase (Tx) and recovery phase (Rec.) were determined for each parameter in every experiment, and the mean values were presented as the percentage of control means. Normalized data for adult heart experiments are summarized in Tables B-1 to B-13 (Appendix B).

4.2.2.5 Statistical Analysis

Results were analyzed by one-way analysis of variance (SuperANOVA, Abacus Concepts Inc., 1989). If the analysis of variance demonstrated significant results, appropriate *post hoc* tests (Dunnett's two-tailed multiple comparison procedure) were used to compare the treatment means to the control mean (Ludbrook, 1991). Correlation between dose and incidence of ectopic beats was determined by the standard least-squares regression technique. The level of significance was accepted as $p < 0.05$.

4.3 RESULTS

4.3.1 Mechanical Parameters

4.3.1.1 Coronary flow

Coronary flow (CF) was maintained at levels at least 85% of baseline throughout the experimental protocol in all control experiments (Appendix B). CH 100 µg/ml (7.5%; $p<0.05$ *vs.* control) and TCE 100 µg/ml (8%; $p<0.05$ *vs.* control) produced modest reductions in CF in the treatment phase and recovery phase, respectively (Figure 4-1). CH 200 µg/ml ($p<0.001$ *vs.* control) and TCA 50-150 µg/ml ($p<0.01$ *vs.* control) caused reductions of about

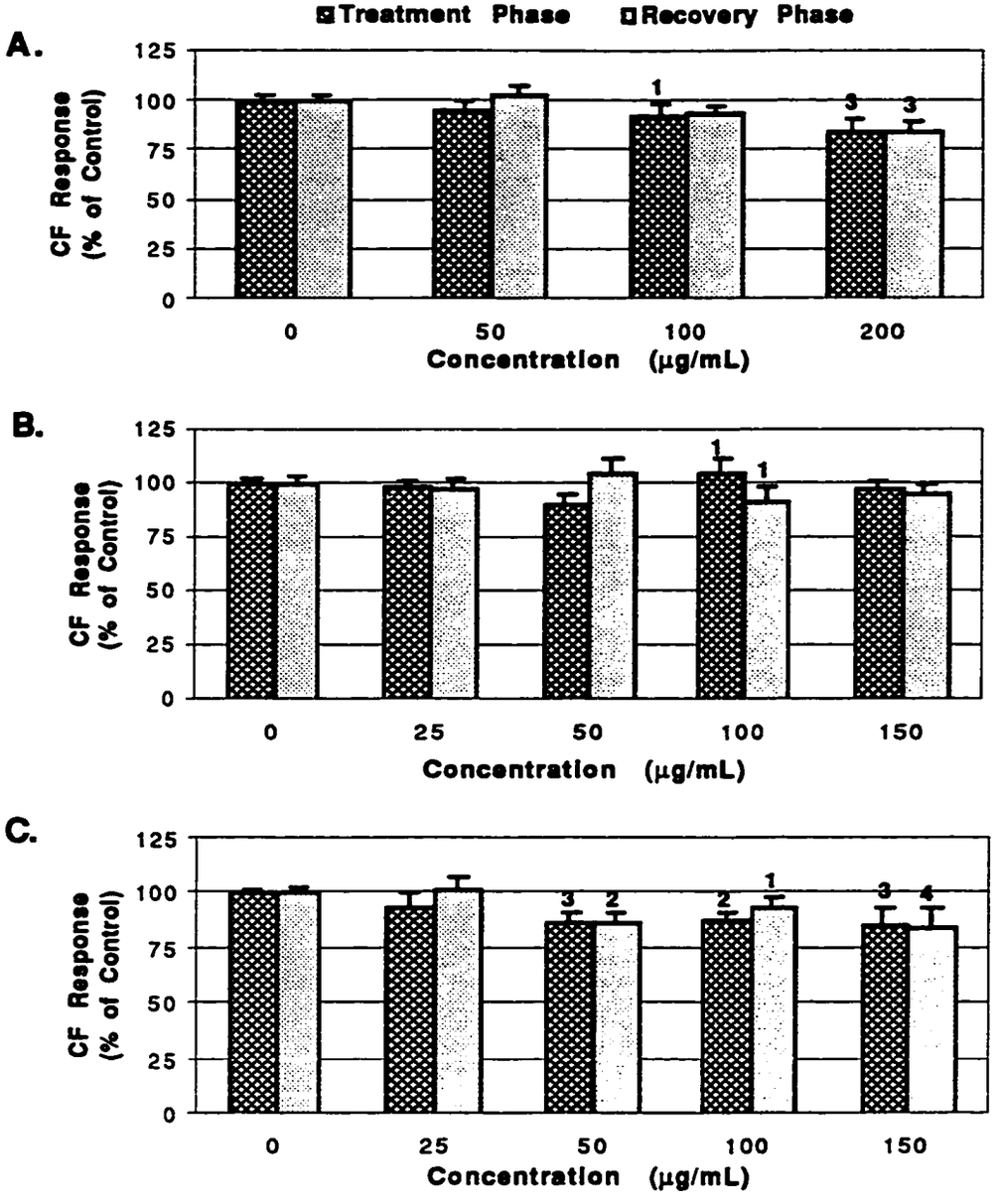


Figure 4-1. The effects of (A) chloral hydrate (0, 50, 100, 200 µg/ml), (B) trichloroethanol (0, 25, 50, 100, 150 µg/ml) and (C) trichloroacetic acid (0, 25, 50, 100, 150 µg/ml) on coronary flow (CF Response) in isolated, perfused adult rabbit hearts. (Results are expressed as per cent of control values for treatment and recovery phases. Error bars indicate standard error of the group means. $^{1}p<0.05$; $^{2}p<0.01$; $^{3}p<0.001$; $^{4}p<0.0001$ *versus* respective control values.)

approximately 15% in CF in the treatment phase, which were not restored in the recovery phase.

4.3.1.2 Myocardial oxygen consumption

Every treatment (with the exception of TCE 25 μg/ml) produced statistically significant, dose-related reductions in myocardial oxygen consumption (MVO_2) (Figure 4-2). TCE administration (150 μg/ml) produced a reduction of 30% ($p<0.01$ *vs.* control). Maximal impairment of MVO_2 was noted with CH (200 μg/ml, $p<0.001$ *vs.* control) and TCA (150 μg/ml, $p<0.01$ *vs.* control) administration, each producing 32% reductions in oxygen consumption. Recovery of oxygen consumption was minimal for low to moderate concentrations of CH, TCE and TCA, while the remainder of the drug treatments remained significantly lower than controls during the recovery phase.

4.3.1.3 Left ventricular contractility

Treatment with CH, TCE, and TCA produced significant impairment of myocardial contractility, as measured by dP/dT (Figure 4-3) . CH 50-200 μg/ml caused reductions in contractility of approximately 30-33% ($p<0.01$ *vs.* control). Recovery of contractility failed to occur in the CH 200 μg/ml treatment group ($p<0.01$). TCE induced a profound dose-dependent depression in contractility, with the highest concentration (150μg/ml) producing a 40% reduction of dP/dT ($p<0.001$). However, pre-treatment (control) levels of dP/dT were re-established for all but 50 μg/ml TCE. TCA 50 and 150 μg/ml produced significant reductions in myocardial contractility, depressing dP/dT by 20% ($p<0.05$ *vs.* control) and 35% ($p<0.001$) respectively. Depressed contractility did not recover for the TCA 50-150 μg/ml groups, and was even further reduced at concentrations of 50 ($p<0.001$ *vs.* control) and 150 μg/ml ($p<0.0001$).

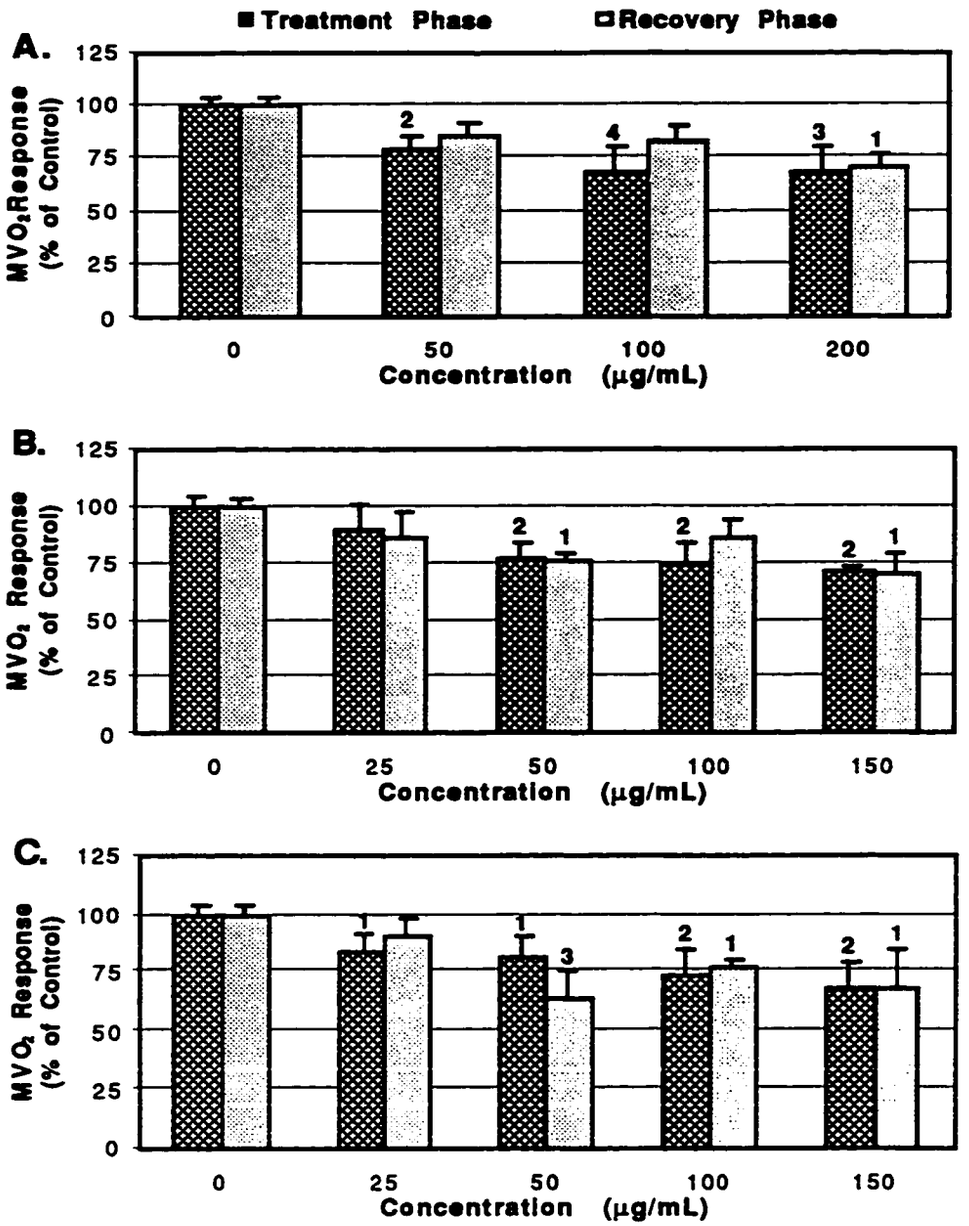


Figure 4-2. The effects of (A) chloral hydrate (0, 50, 100, 200 µg/ml), (B) trichloroethanol (0, 25, 50, 100, 150 µg/ml) and (C) trichloroacetic acid (0, 25, 50, 100, 150 µg/ml) on myocardial oxygen consumption (MVO_2 Response) in isolated, perfused adult rabbit hearts. (Results are expressed as per cent of control values for treatment and recovery phases. Error bars indicate standard error of the group means. $^1p<0.05$; $^2p<0.01$; $^3p<0.001$; $^4p<0.0001$ *versus* respective control values.)

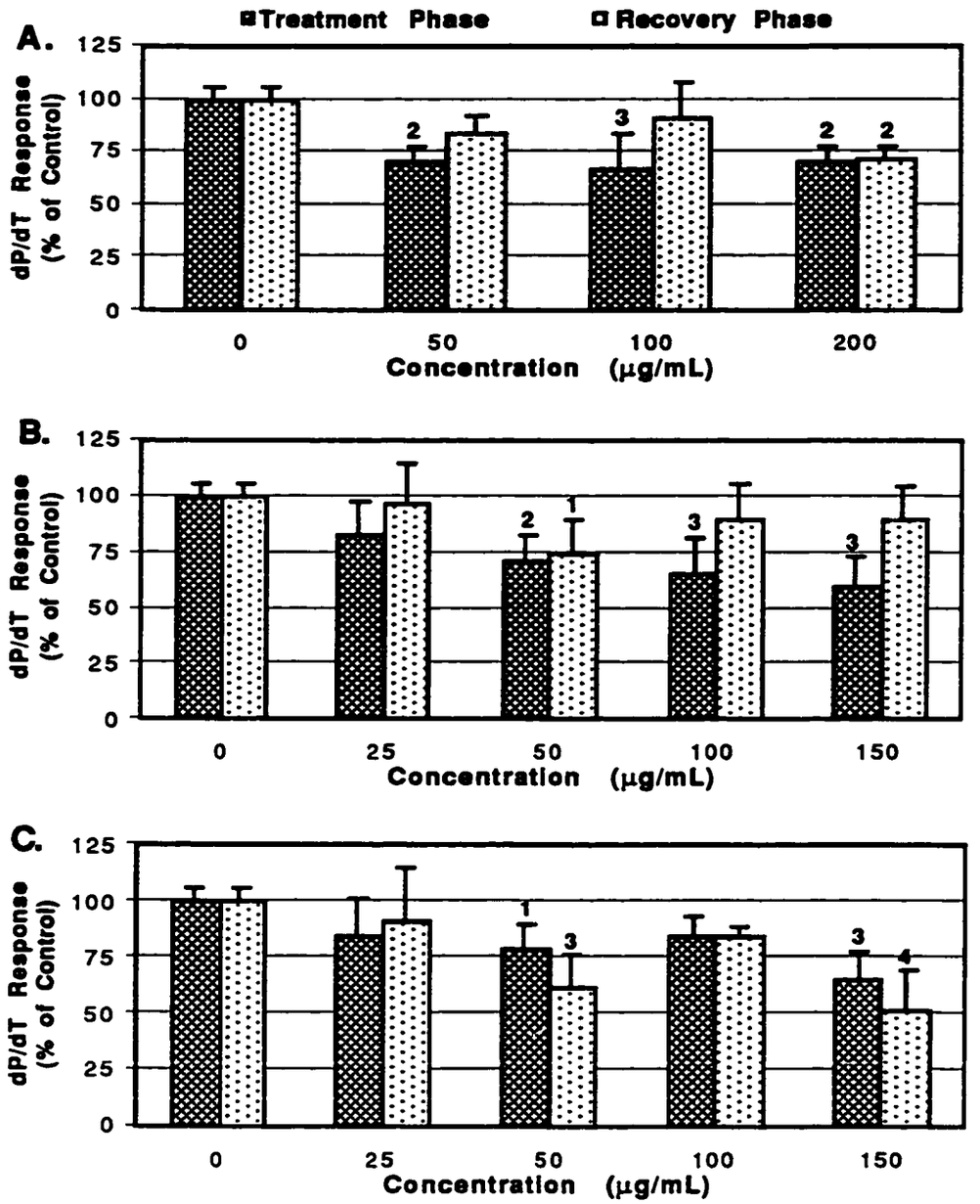


Figure 4-3. The effects of (A) chloral hydrate (0, 50, 100, 200 µg/ml), (B) trichloroethanol (0, 25, 50, 100, 150 µg/ml) and (C) trichloroacetic acid (0, 25, 50, 100, 150 µg/ml) on left ventricular contractility (dP/dT Response) in isolated, perfused adult rabbit hearts. (Results are expressed as per cent of control values for treatment and recovery phases. Error bars indicate standard error of the group means. $^{1}p<0.05$; $^{2}p<0.01$; $^{3}p<0.001$; $^{4}p<0.0001$ *versus* respective control values.)

4.3.2 Electrocardiographic Parameters

4.3.2.1 Heart rate

CH, TCE, and TCA produced significant dose-related reductions in heart rate in the isolated hearts (Table 4-1). CH (50-200 μg/ml) reduced heart rate by 10-20% ($p<0.05$ *vs.* control). Minimal reversal of bradycardia was seen during the recovery phase. At the two highest concentrations, TCE caused moderate bradycardia (heart rate 85% of control, $p<0.01$ *vs.* control). Heart rates were also reduced during the recovery phase for the 25 μg/ml and 150 μg/ml treatments (79% and 68% of control, respectively; $p<0.0001$). TCA treatment produced significant bradycardia at concentrations of 50-150 μg/ml (heart rates 85-77% of control; $p<0.05$). Recovery of normal heart rate was not observed at the 50 and 150 μg/ml concentrations ($p<0.01$ *vs.* control).

4.3.2.2 Atrio-ventricular conduction (P-R interval)

Table 4-1 illustrates the effects of CH, TCE and TCA on atrio-ventricular conduction, as measured by the P-R interval of the ECG. CH (50-200 μg/ml) and TCE (25,100 and 150 μg/ml) markedly delayed atrio-ventricular conduction, with P-R intervals ranging from 115 to 125% of control values ($p<0.05$). However, the conduction delay persisted only in the TCE 150 μg/ml group ($p<0.01$ *vs.* control). TCA profoundly delayed atrio-ventricular conduction, increasing the P-R interval to 125 to 141% of control values ($p<0.001$). The conduction delays produced by TCA 50-150 μg/ml persisted throughout the recovery phase, remaining at between 123 and 162% of control values ($p<0.05$).

4.3.2.3 Intra-ventricular conduction (QRS duration)

Table 4-1 shows the effects of CH, TCE and TCA on intra-ventricular conduction, as measured by the duration of the QRS complex. CH 200 μg/ml and TCE 25 μg/ml produced persistent conduction delays, with QRS durations increased to 115-119% of control levels ($p<0.05$) throughout the

Table 4-1. Effects of chloral hydrate, trichloroethanol and trichloroacetic acid on electrocardiographic parameters in the isolated perfused adult rabbit heart.[§]

ECG Parameter		Control	Chloral hydrate (µg/ml)			Trichloroethanol (µg/ml)				Trichloroacetic acid (µg/ml)			
			50	100	200	25	50	100	150	25	50	100	150
Heart Rate	Tx	100.0 [1.3]	89.1[a] [5.1]	83.9[b] [5.4]	79.8[c] [3.1]	90.3 [3.8]	91.0 [8.3]	85.1[b] [11.0]	85.7[b] [4.7]	90.2 [4.1]	85.6[a] [4.1]	78.4[d] [8.1]	77.0[d] [5.8]
	Rec.	100.0 [1.8]	96.4 [4.1]	87.7[a] [5.0]	82.7[c] [4.7]	79.3[d] [7.2]	98.5 [3.1]	91.2 [6.4]	68.0[d] [10.4]	94.6 [6.2]	83.0[c] [7.2]	93.3 [6.2]	83.9[b] [6.5]
P-R interval	Tx	100.0 [1.6]	116.0[a] [7.3]	123.1[c] [11.3]	117.2[a] [3.7]	116.0[a] [4.1]	109.4 [7.2]	124.4[c] [5.5]	115.1[a] [4.4]	126.0[c] [7.2]	141.4[d] [6.3]	141.4[d] [9.5]	130.6[d] [4.8]
	Rec.	100.0 [3.2]	100.1 [6.5]	106.1 [5.4]	100.3 [6.2]	99.0 [4.4]	99.2 [9.5]	114.1 [7.5]	125.2[b] [15.4]	104.2 [6.6]	161.5[d] [10.1]	123.3[a] [12.9]	153.2[d] [17.6]
QRS Dur'n	Tx	100.0 [3.4]	110.7 [4.3]	108.4 [2.0]	117.3[b] [7.5]	118.9[b] [6.3]	106.2 [2.1]	111.8[a] [4.0]	108.7 [6.0]	118.2[b] [4.8]	123.2[d] [6.5]	118.4[b] [6.3]	109.6[a] [3.4]
	Rec.	100.0 [3.4]	104.6 [4.9]	105.2 [4.4]	115.5[a] [3.6]	114.3[a] [5.9]	106.5 [3.2]	110.8 [3.1]	103.5 [4.0]	112.5[a] [9.6]	126.9[d] [6.3]	122.5[c] [9.9]	121.2[c] [7.7]

[§] Results are expressed as percent of respective control values; [S.E.]
Abbreviations: Tx=treatment phase; Rec.=recovery phase; Dur'n=duration

[a] $p<0.05$, [b] $p<0.01$, [c] $p<0.001$, [d] $p<0.0001$ *vs.* controls

experimental protocol. TCE 100 µg/ml caused a modest increase in the QRS duration (111%, $p<0.05$ *vs.* control) in the treatment phase only. TCA induced significant and persistent intra-ventricular conduction delays at all concentrations tested. During the treatment phase, the QRS duration ranged from 110 to 123% of control values ($p<0.05$), and QRS duration remained between 112 and 127% of the control levels for the recovery phase ($p<0.05$).

4.3.2.4 Cardiac arrhythmias

The incidence of ectopic beats arising in either the atria or ventricles was determined, and the results are illustrated in Figure 4-4. CH significantly increased the number of ectopic beats per minute in a dose-dependent fashion in the treatment ($r^2=0.9824$; $p=0.0002$) and recovery ($r^2=0.9164$; $p=0.0012$) phases. While no clear dose-response relationship can be detected for TCE and TCA treatments at the concentrations tested, it is apparent that these compounds significantly increased the number of conduction disturbances per minute in both treatment and recovery phases ($p<0.05$ *vs.* controls). It is possible that a dose-response relationship between TCE or TCA treatment and the incidence of ectopic beats exists for concentrations less than 25 µg/ml. Overall, the apparent potency for initiating ectopic beats was TCA>TCE>CH.

The cardiac arrhythmias observed during drug treatments included those of atrial, junctional and ventricular origin. Table 4-2 outlines the various ECG abnormalities detected for each of the treatment groups. Brady-tachy syndrome (a manifestation of the sick sinus syndrome) was observed only with TCE (50 and 100 µg/ml) treatment. Disturbances of the atrial and ventricular conduction pathways were quite common in all treatment groups. Type I second-degree atrioventricular block (2° AV block - Type I) predominated in CH and TCE treatments, while type II 2° AV block and the more severe third-degree AV block occurred principally in the TCA treatments. Various bundle branch blocks were common in all of the treatments. Premature atrial contractions (PAC's) and paroxysmal supraventricular tachycardia (PSVT)

were observed with about the same frequency in all treatments. Junctional escape beats (JEB's) and ectopic pacemakers were observed only in TCE and TCA treated hearts. Premature ventricular contractions (PVC's) were seen in all treatment groups. While PVC's occurred during control protocols, the occurrences were infrequent and isolated. Ventricular tachycardia (v. tachycardia) and ventricular fibrillation (v. fibrillation; considered the endpoint of arrhythmia in this protocol) were observed only in TCA treated hearts. Electrocardiographic tracings illustrating specific cardiac arrhythmias are located in Appendix B (Figures B-1 to B-5).

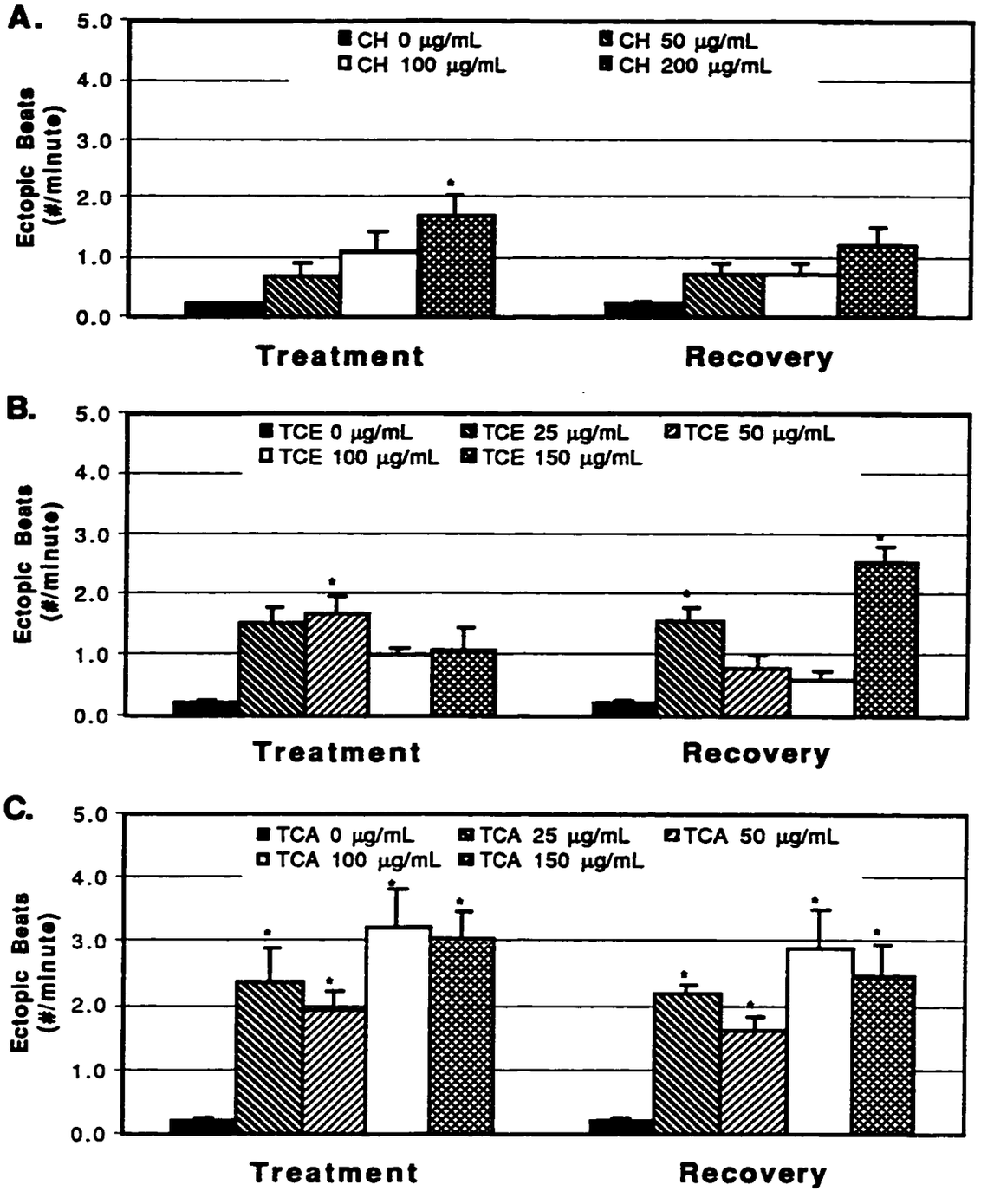


Figure 4-4. Incidence of ectopic beats in (A) chloral hydrate (0, 50, 100, 200 µg/ml), (B) trichloroethanol (0, 25, 50, 100, 150 µg/ml) and (C) trichloroacetic acid (0, 25, 50, 100, 150 µg/ml) treated isolated, perfused adult rabbit hearts. (Results are expressed as number of ectopic beats observed per minute in treatment and recovery phases. Error bars indicate the standard error of the group means. *$p<0.05$ *versus* the respective control values.)

Table 4-2. Cardiac arrhythmias occurring in the isolated, perfused adult rabbit heart during control, chloral hydrate, trichloroethanol or trichloroacetic acid treatment.

Cardiac Arrhythmias	Control	Chloral Hydrate (μg/mL)			Trichloroethanol (μg/mL)				Trichloroacetic Acid (μg/mL)			
		50	100	200	25	50	100	150	25	50	100	150
Premature ventricular contraction	◊	◊	◊	◊	◊◊	◊	◊	◊	◊	◊◊	◊	◊
Ventricular tachycardia									◊	◊	◊	◊
Torsade de pointes									◊	◊	◊	◊
Ventricular fibrillation										◊		◊
Premature atrial contraction		◊	◊	◊◊	◊	◊		◊	◊◊	◊◊		◊
Premature supra-ventricular tachycardia			◊	◊	◊	◊	◊	◊		◊		◊
Sick sinus syndrome						◊	◊					
2° Atrioventricular block:												
Type I			◊	◊	◊	◊	◊	◊	◊			
Type II			◊	◊	◊			◊	◊	◊◊	◊◊	◊◊
3° Atrioventricular block									◊	◊◊	◊	◊
Junctional escape beat					◊	◊			◊	◊	◊	◊
Ectopic pacemaker					◊	◊	◊	◊		◊	◊	
Bundle branch block		◊	◊	◊	◊	◊	◊	◊		◊	◊	◊

◊ : denotes occurrence in at least one experiment
◊◊: denotes occurrence in at least four experiments

4.4 DISCUSSION AND CONCLUSIONS

This study was undertaken to investigate the effects of CH, TCE and TCA on the isolated perfused adult rabbit heart in an effort to elucidate the direct cardiotoxic potential of these agents. Species variation in the magnitude of response to pharmacologic agents is quite common; however, the therapeutic plasma concentration of a particular agent observed in animals is usually comparable to that seen in humans (Baggot, 1992). For this reason, concentrations of CH, TCE and TCA selected for this investigation were based on the range of plasma concentrations observed in human pharmacokinetic studies (Hindmarsh *et al.*, 1991; Mayers, 1992). The perfused heart preparation is a valuable tool for the evaluation of interventions that, in the intact organism, may have direct and/or indirect actions on the heart (Henry *et al.*, 1977). Since the rabbit heart has been widely employed for studies of cardiac function (Roberts, 1991), and since drugs tend to induce similar qualitative effects in different species (Weiner and Newberne, 1977), this model system was selected for this investigation.

The patency of the coronary vasculature was not detrimentally affected by the experimental protocol itself, as evidenced by the maintenance of CF throughout the control protocols (Appendix B, Table B-1). Only CH (200 µg/ml) and TCA (50-150 µg/ml) produced persistent reductions in CF, which may be indicative of irreversible damage to the coronary vasculature. This effect is not surprising, given the well-known caustic nature of these compounds (Windholz, 1983). There is a close relationship, even in the isolated heart, between the level of myocardial metabolic activity and the magnitude of CF (Berne and Levy, 1993b; Neely *et al.*, 1967). Thus, reduced CF would be predicted to depress metabolic activity of the myocardium. Decreased CF can also produce diminished washout of coronary vasculature, resulting in an accumulation of metabolic products (Neely *et al.*, 1967). It could be assumed that drugs (CH, TCE and TCA) would also accumulate in the ischemic region, providing increased opportunities for metabolism and/or toxic effects.

It would be predicted in an isolated heart system that a decrease in CF (ischemia) would result in tissue hypoxia (Berne and Levy, 1993b). In addition, it has been shown that CH impairs the rate-limiting enzymes of both aerobic and anaerobic metabolism in the coronary vasculature (Trulson and Ulissey, 1987). Relative tissue hypoxia, combined with inhibition of coronary metabolism, would be predicted to diminish myocardial oxygen consumption. This was observed with CH, TCE and TCA administration, all of which caused significant, dose-related reductions in myocardial oxygen consumption. The MVO_2 remained significantly depressed in the recovery phase for most treatments. It has been estimated that irreversible damage to the myocardium occurs after less than an hour of ischemia (Neely *et al.*, 1967). Thus, irreversible myocardial damage is likely after CH, TCE or TCA treatments in this protocol.

Ventricular contracture is a characteristic response of the myocardium following ischemic insult. An increase of 5 mmHg in left ventricular end-diastolic pressure is considered to be a reasonable indication of ventricular contracture (Quantz *et al.*, 1992). Ventricular contracture was observed in the recovery phase for certain experiments, including: CH 50, 100 µg/ml; TCE 25, 50, 150 µg/ml; TCA 25, 50, 100, 150 µg/ml (data not shown). This phenomenon was not observed in a consistent fashion for any of the treatments; however, the occurrence of contracture during the recovery phases of these experiments is suggestive of the ischemia/reperfusion phenomenon.

The rate of rise of left ventricular pressure (dP/dT) is affected by preload, afterload, contractility and heart rate (Prasad *et al.*, 1991). However, this parameter constitutes a reasonable index of myocardial contractility in the isolated heart system since preload and afterload are fixed (Berne and Levy, 1993a; Henry *et al.*, 1977; Riggs *et al.*, 1986). The dP/dT has been found to correlate with left ventricular oxygen consumption (Berne and Levy, 1993b); thus, impairment of myocardial contractility by CH, TCE and TCA would be anticipated. However, dP/dT was depressed to a greater extent than MVO_2 in all treatments. Additionally, improvement of contractility occurred during the

recovery phase for CH (50 and 100 μg/ml), TCE (25, 100 and 150 μg/ml) and TCA (25 μg/ml) treatments. It is unlikely, then, that the reduction in MVO_2 is completely responsible for the impairment of myocardial contractility in either the treatment or recovery phases of the protocols (Parrish *et al.*, 1987).

CH, TCE and TCA produced significant dose-related reductions in heart rate in the isolated hearts. In all cases, heart rate was reduced to a greater extent than coronary flow; therefore, these compounds can be presumed to cause a direct bradycardic effect. The most likely causes of bradycardia would be sinus bradycardia, atrio-ventricular block (second- or third-degree) or atrio-ventricular junctional escape rhythm. Several instances of TCE-induced bradycardia occurred alternately with tachycardia. This "brady-tachy syndrome" is a subset of the sick sinus syndrome in which tachyarrhythmias occur in an alternating fashion with bradyarrhythmias (Goldberger and Goldberger, 1990b).

All three compounds caused significant impairment of atrio-ventricular and intra-ventricular conduction, which is associated with the development of re-entry and resultant arrhythmias (Katz, 1992). TCA produced the most significant and persistent conduction delays of both atrio-ventricular and intra-ventricular origin. Ventricular tachycardia (including torsade de pointes) and ventricular fibrillation were observed only in TCA-treated hearts. Torsade de pointes has been previously observed in a patient who ingested an over-dose of CH. TCA was suggested to have been the causative factor leading to the arrhythmia, as the prolonged arrhythmia seemed to parallel the long half-life of TCA (Young *et al.*, 1986).

With the exception of ectopic beats, all results have been expressed as time-averaged values encompassing the treatment and recovery phases, respectively. This approach was used to enable the assessment of the differences of the physiological parameters from their control values over time. The cardiovascular effects of anesthetic agents are known to be time-dependent; thus, it is believed that measurements observed at a single time point can be misleading in their study. Therefore, the investigation of such

agents is thought to be incomplete without including the effects over time in the overall assessment of toxicity (Eger *et al.*, 1970). Since CH and its active metabolite TCE appear to exert their cardiotoxic effects in a fashion analogous to halothane (Brown and Cade, 1980), the effects exerted over time may be of importance in toxicity assessment. Use of time-averaged data for treatment and recovery phases also allowed for the assessment of the overall effects of the drug/metabolite, minimizing the impact of normal physiological variation over time on the interpretation of the results. This approach should provide more relevant information than the assessment of various effects for each ten minute interval (Urquhart, 1981).

Certain investigators have undertaken limited examinations of the potential of CH to produce cardiac toxicity. An early study of the effects of chloral hydrate on the hearts of both dogs and horses appeared to show a primarily vagal mechanism in the development of arrhythmias (Gross and Hoff, 1968). Others examined the arrhythmogenicity of trichloroethylene and its metabolites (TCE and TCA). They concluded that trichloroethylene, rather than its metabolites, sensitized the myocardium to epinephrine-induced arrhythmias (White and Carlson, 1981). No significant deterioration of a rat heart preparation was found when CH (300 mg/kg) was used as the anesthetic agent prior to excision of the hearts (Riggs *et al.*, 1986). Recently, histochemical and enzymatic alterations in the heart following acute CH administration were examined. The results of this investigation indicate that non-toxic doses of CH can impair the metabolism of coronary vascular smooth muscle by attacking key cardiac enzymes. Unfortunately, no attempt was made to determine metabolite concentrations (Trulson and Ulissey, 1987). None of the preceding investigations examined the specific cardiotoxic potential of CH and its two major metabolites TCE and TCA on both mechanical and electrocardiographic parameters.

The present investigation has demonstrated that CH, TCE and TCA all have general cardiac depressant effects in the isolated perfused adult rabbit heart at concentrations routinely encountered in clinical situations. The

depressant effects of these agents on the CF, MVO_2, dP/dT, and HR appear to be largely dose-related. More importantly, TCA produced severe and persistent conduction disturbances, resulting ultimately in ventricular tachycardia and/or ventricular fibrillation at all concentrations tested. Ventricular tachyarrhythmias (ventricular fibrillation or a sustained type of ventricular tachycardia) are characteristic ECG patterns associated with cardiac arrest (Goldberger and Goldberger, 1990a). These findings are especially significant when one considers the persistence of TCA in humans following administration of CH (Hindmarsh *et al.*, 1991; Müller *et al.*, 1974; Sellers *et al.*, 1978). In situations of increased production and/or reduced clearance of TCA, there exists the possibility for significant cardiac toxicity, including the development of potentially life-threatening arrhythmias, to occur following CH administration.

5. EFFECTS OF CHLORAL HYDRATE & ITS METABOLITES ON THE ISOLATED PERFUSED NEONATAL RABBIT HEART

5.1 INTRODUCTION

While chloral hydrate (Noctec®; CH) does not currently enjoy its former extensive use in the general population, it is still one of the most commonly used sedatives in pediatric practice (American Academy of Pediatrics, 1993; Cook *et al.*, 1992; Green, 1993). CH is used as a maintenance sedative for the management of agitation in critically ill newborns, especially those treated with positive pressure ventilation (Hartley *et al.*, 1989; Mayers *et al.*, 1991; Noerr, 1992). CH is also used in acute situations to provide sedation for non-invasive pediatric diagnostic procedures and in pediatric dentistry (Cook *et al.*, 1992; Lichenstein *et al.*, 1993; Marx *et al.*, 1993; Zarifi *et al.*, 1995).

Chloral hydrate is rapidly metabolized in adult subjects both by reduction to trichloroethanol and by oxidation to trichloroacetic acid. TCE is further conjugated with glucuronic acid prior to excretion in the urine (Breimer *et al.*, 1974; Sellers *et al.*, 1978). When administered to healthy adult volunteers, CH is nearly undetectable within minutes of administration (Breimer *et al.*, 1974; Gorecki *et al.*, 1990). However, metabolism of CH in the neonate is characterized by immaturity of hepatic and renal elimination processes. Contrary to the situation in the adult, CH was detectable for several hours after a single oral dose (50 mg/kg) in neonates and young children (Mayers *et al.*, 1991). The elimination half-lives of both TCE (30-40 h) (Gershanik *et al.*, 1981; Hindmarsh *et al.*, 1991; Mayers *et al.*, 1991) and TCA (>6 days) (Hindmarsh *et al.*, 1991; Mayers *et al.*, 1991) are substantially prolonged in neonates when compared to adult volunteers (8-12 h and 67-100

h, respectively) (Breimer *et al.*, 1974; Sellers *et al.*, 1972b; Sellers *et al.*, 1978). It has been established that neonates and young children tend to accumulate TCE and TCA, especially in multiple-dosing situations (Hindmarsh *et al.*, 1991). Of particular concern in the neonatal population is the persistence of TCA, a potentially toxic metabolite, even after a single oral dose (Hindmarsh *et al.*, 1991; Mayers *et al.*, 1991).

Previous investigations in this laboratory have demonstrated that CH, TCE and TCA, in clinically achievable concentrations, are cardiac depressants and have definite arrhythmogenic potential (Fandrey *et al.*, 1992; Chapter 4). However, the cardiotoxic and arrhythmogenic potentials of CH, TCE and TCA remain unknown in the vulnerable neonatal population. The present investigation was performed to elucidate whether CH and/or its metabolites are involved in cardiac toxicity in the isolated, perfused neonatal rabbit heart.

5.2 EXPERIMENTAL

5.2.1 Materials

5.2.1.1 Chemicals

Chloral hydrate was obtained from Bristol-Myers Squibb Canada, Inc. (Montreal, PQ), trichloroacetic acid was purchased from Fluka Chemicals, Terochem Laboratories Ltd. (Edmonton, AB), and trichloroethanol was obtained from Aldrich Chemical Co. (Milwaukee, WI). All other chemicals were purchased from either Sigma Chemical Co. (St. Louis, MO) or BDH Chemicals (Toronto, ON). All chemicals used were of reagent grade.

5.2.1.2 Animals

Newborn New Zealand white rabbits (7-10 day) of either sex were obtained from the licensed breeding facility at the Animal Resources Centre, University of Saskatchewan (see Appendix A, Table A-2). Animals were obtained on the morning of the experiment, and were housed in a warm (30-35°C), dark environment prior to use. Animals used in this research were

cared for in accordance with the recommendations of the Canadian Council on Animal Care (CCAC).

5.2.2 Methods

5.2.2.1 Experimental Protocol

5.2.2.1.1 Heart Perfusion

Animals were sacrificed by decapitation according to accepted CCAC protocols. Anesthetic exposure was avoided to prevent any potential for interaction with the subsequent drug (CH, TCE, or TCA) treatments. The hearts were rapidly excised and placed immediately in ice-cold perfusion buffer. The aorta was cannulated quickly (within 1 minute) and Langendorff perfusion (Langendorff, 1895) was initiated at a high flow rate to clean the coronary arteries. Once the arteries were sufficiently flushed, the heart was perfused as described previously (Prasad *et al.*, 1992b). Retrograde perfusion was maintained at a constant perfusion pressure of 100 cmH_2O with a bicarbonate buffer, pH 7.4, of the following composition (in mM): sodium chloride, 154.0; potassium chloride, 5.63; sodium bicarbonate, 5.95; calcium chloride, 2.16; and glucose, 5.55. The buffer passed through an in-line filter prior to heart perfusion, was equilibrated with a gas mixture of 95% O_2 and 5% CO_2 and was maintained at 37° C.

A small incision was made in the left atrium, and a balloon catheter was introduced into the left ventricle through the mitral valve. The balloon was filled with a volume of saline solution sufficient to raise the left ventricular end-diastolic pressure (LVEDP) to 20 mmHg. The catheter was attached to a Gould pressure transducer (Gould Inc. Cardiovascular Products Division, Oxnard, CA) and a Beckman R411 dynograph recorder. Lead II ECG was monitored and recorded simultaneously. The heart was perfused at a rate of 8.4 ± 0.4 mL/minute, and was allowed to stabilize for at least 45 minutes before any interventions.

5.2.2.1.2 Myocardial Assessment

The mechanical parameters determined from the recordings were: left ventricular developed pressure (LVDP = LVSP - LVEDP; mmHg); dP/dT (mmHg/s; a reflection of myocardial contractility); and index of myocardial oxygen consumption (MVO_2 = LVSP x HR; mmHg/min). LVDP and its first derivative (dP/dT) were used to assess left ventricular compliance and contractility, respectively, in the isolated perfused heart. Coronary flow (CF; mL/min) was determined by collecting the perfusate over one minute.

Lead II ECG was monitored for the assessment of cardiac arrhythmias. The diagnostic ECG parameters were: heart rate (HR; beats/min); P-R interval (seconds; an indication of atrio-ventricular conduction); QRS duration (seconds; an indication of conduction within the ventricles); ectopic beats (atrial and ventricular); ventricular tachycardia; and ventricular fibrillation (which was considered to be the end point of arrhythmia). These parameters were monitored every 10 minutes throughout the experimental protocol, and more frequently if the situation warranted close monitoring.

5.2.2.1.3 Drug Administration

Drug solutions (CH 50, 100, 200 μg/ml or 0.30, 0.61, 1.2 mM; TCE 25, 50, 100, 150 μg/ml or 0.17, 0.34, 0.67, 1.0 mM; and TCA 25, 50, 100, 150 μg/ml or 0.15, 0.31, 0.61, 0.92 mM) were prepared fresh daily using the buffer as solvent. The perfusion apparatus was connected such that separate reservoirs for pure buffer and drug/buffer solutions could be employed. Drug administration via the buffer solution was initiated after stabilization of the heart and continued for 60 minutes (treatment phase). Following the treatment phase, the perfusion was switched back to pure buffer and the heart was monitored for an additional 60 minutes (recovery phase). Ten control experiments were performed, while drug interventions were repeated 6-7 times for each dosing level (see Appendix A, Table A-2).

5.2.2.1.4 Expression of Results

Individual experimental parameters were time-averaged over the course of the treatment and recovery phases (responses were calculated every 10 minutes during the protocol), by calculating the areas under the response *vs.* time curve (AUC's) using Lagrange interpolation (Rocci and Jusko, 1983). The AUC's for the treatment phase (Tx) and recovery phase (Rec.) were determined for each parameter in every experiment, and the mean values were presented as the percentage of control means. Normalized data for neonatal heart experiments are summarized in Tables C-1 to C-13 (Appendix C).

5.2.2.1.5 Statistical Analysis

Results were analysed by one-way analysis of variance (SuperANOVA, Abacus Concepts Inc., 1989). If the analysis of variance demonstrated significant results, appropriate *post hoc* tests (Dunnett's two-tailed multiple comparison procedure) were used to compare the treatment means to the control mean (Ludbrook, 1991). Correlations between dose and incidence of ectopic beats were determined by the standard least-squares regression technique. The level of significance was accepted as $p < 0.05$.

5.3 RESULTS

5.3.1 Mechanical Parameters

5.3.1.1 Coronary flow

Coronary flow was maintained at levels at least 90% of baseline throughout the experimental protocol in all control experiments (Appendix C, Table C-1). Only CH 50 μg/ml and TCA 100 μg/ml had significant effects on the coronary flow during the treatment phase, producing 32% ($p<0.0001$ *vs.* control) and 12% ($p<0.05$ *vs.* control) reductions in CF, respectively. In all other treatment groups, the CF was between 89 and 99% of control values (Figure 5-1).

5.3.1.2 Myocardial oxygen consumption

Figure 5-2 illustrates the effects of CH, TCE and TCA on myocardial oxygen consumption (MVO_2) during the treatment and recovery phases of the experimental protocol. TCE treatment caused no significant alterations in MVO_2. CH administration appeared to cause a slight trend towards reduced MVO_2 during the treatment phase. This effect, however, was significant only for the 50 μg/ml treatment which produced an 18% reduction in MVO_2 ($p<0.05$ *vs.* control). During the recovery phase of the CH 50 μg/ml treatment, MVO_2 was significantly increased (130% of control; $p<0.05$). Both the CH 100 and 200 μg/ml groups showed significant reductions in MVO_2 (18 and 20% respectively; $p<0.05$) during their recovery phases. TCA 100 μg/ml produced a 15% decrease ($p<0.05$ *vs.* control) in MVO_2 during the treatment phase; MVO_2 was further reduced (68% of control; $p<0.001$) in the recovery phase.

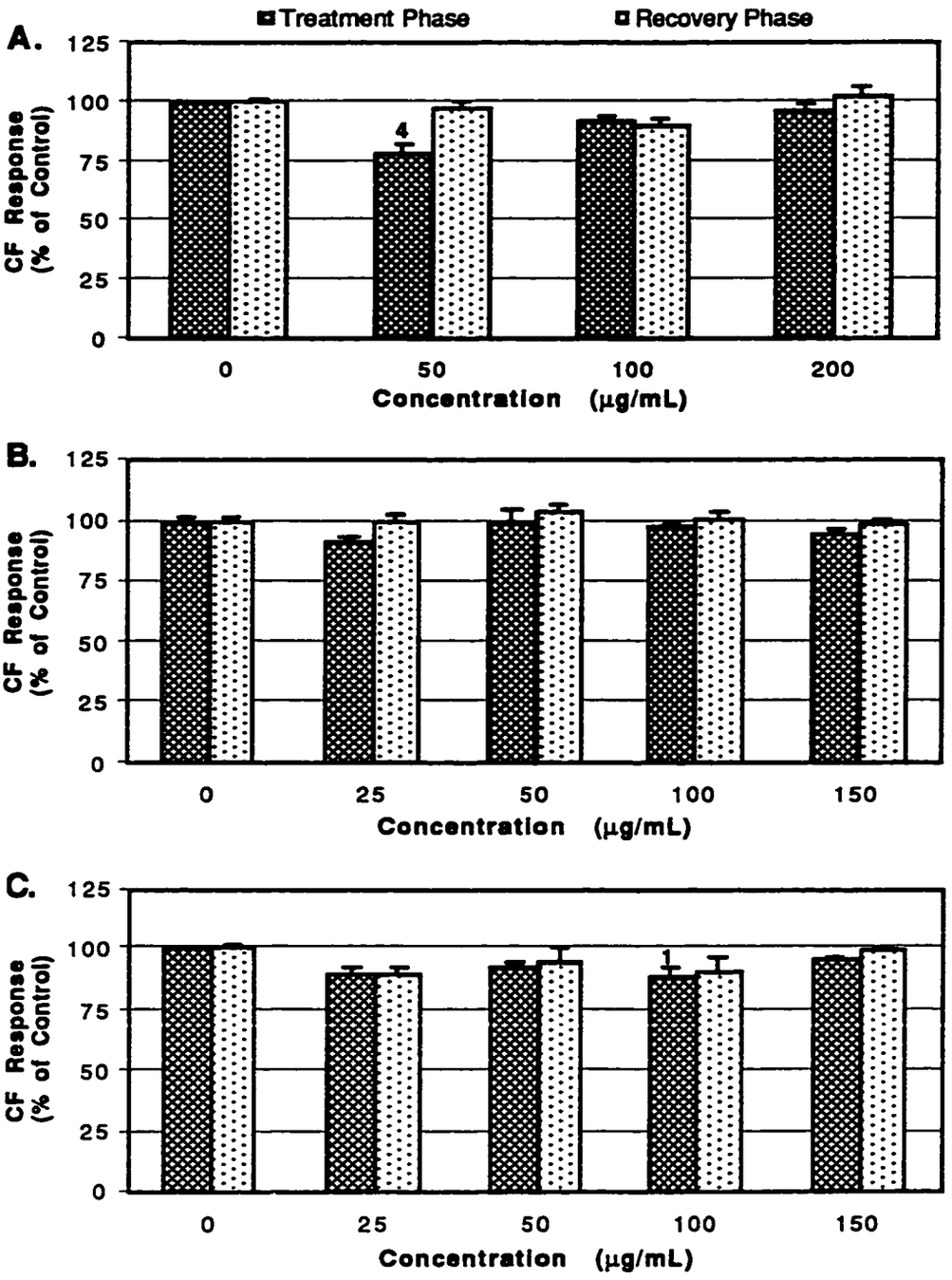


Figure 5-1. The effects of (A) chloral hydrate (0, 50, 100, 200 µg/ml), (B) trichloroethanol (0, 25, 50, 100, 150 µg/ml) and (C) trichloroacetic acid (0, 25, 50, 100, 150 µg/ml) on coronary flow (CF Response) in isolated, perfused neonatal rabbit hearts. (Results are expressed as per cent of control values for treatment and recovery phases. Error bars indicate standard error of the group means. $^{1}p<0.05$; $^{2}p<0.01$; $^{3}p<0.001$; $^{4}p<0.0001$ *versus* respective control values.)

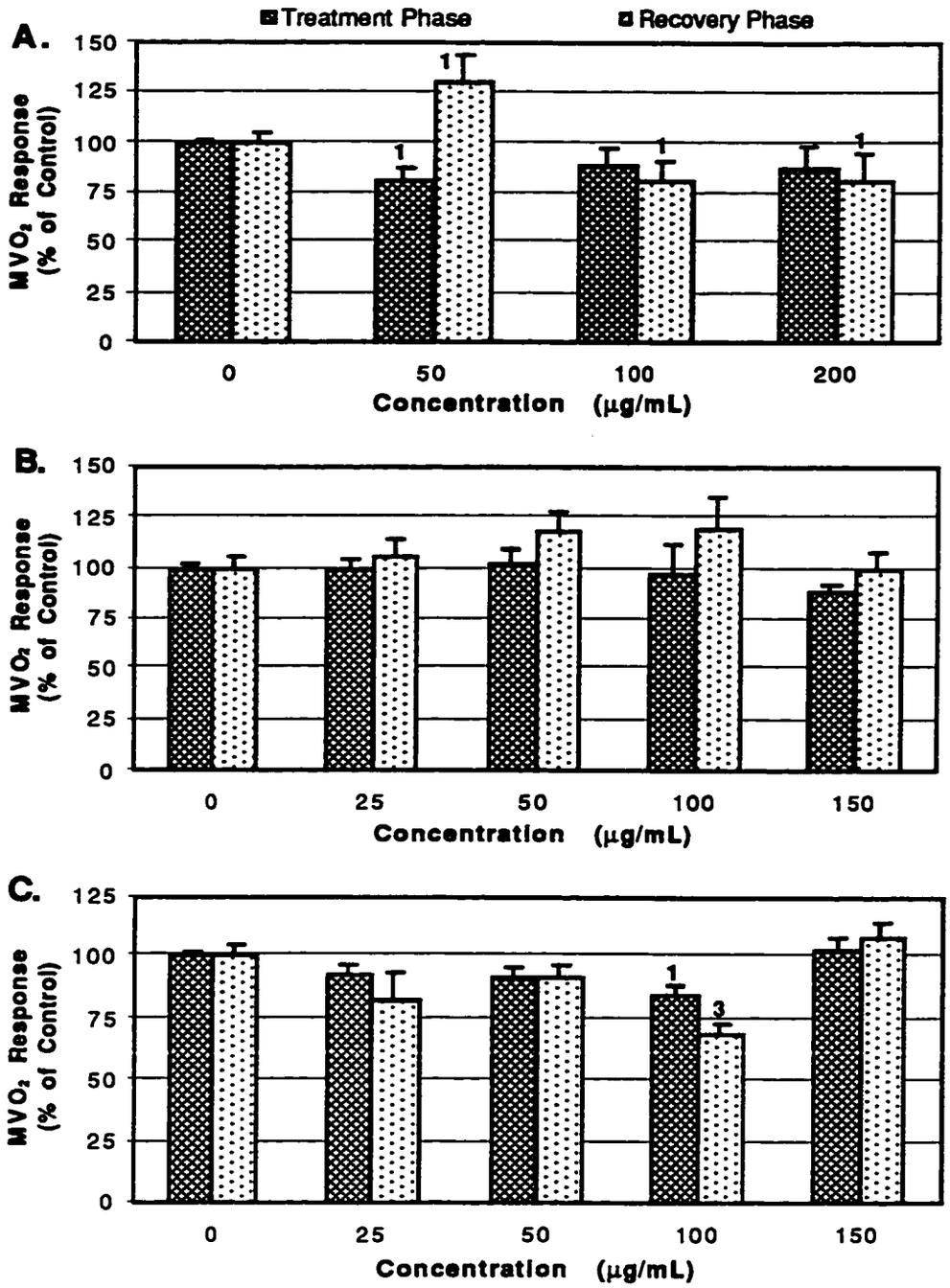


Figure 5-2. The effects of (A) chloral hydrate (0, 50, 100, 200 µg/ml), (B) trichloroethanol (0, 25, 50, 100, 150 µg/ml) and (C) trichloroacetic acid (0, 25, 50, 100, 150 µg/ml) on myocardial oxygen consumption (MVO_2 Response) in isolated, perfused neonatal rabbit hearts. (Results are expressed as per cent of control values for treatment and recovery phases. Error bars indicate standard error of the group means. $^1p<0.05$; $^2p<0.01$; $^3p<0.001$; $^4p<0.0001$ *versus* respective control values.)

5.3.1.3 Left ventricular developed pressure

Left ventricular compliance, as estimated by the left ventricular developed pressure (LVDP), was stable during the treatment phases of all treatment groups. However, the effects of various drug treatments appeared to be manifested during the recovery phase of the protocols (Figure 5-3). CH 100 µg/ml produced a 23% reduction ($p<0.05$ *vs.* control) during the recovery phase. TCE 100 µg/ml produced a significant increase in LVDP (125% of control; $p<0.05$) during the recovery phase. Both TCA 25 and 50 µg/ml groups reduced LVDP by 27 and 30%, respectively ($p<0.05$ and 0.01).

5.3.1.4 Left ventricular contractility

Left ventricular contractility has been assessed by determining the first derivative of the LVDP (dP/dT). Like the LVDP, dP/dT was affected only in the recovery phases of the treatment protocols (Figure 5-4). CH 50 µg/ml and TCE 50 µg/ml caused increases in dP/dT of 17 and 26%, respectively ($p<0.05$ and 0.01 *vs.* control), during their recovery phases. TCA 25 µg/ml produced a reduction in dP/dT of 38% ($p<0.01$ *vs.* control) during the recovery phase.

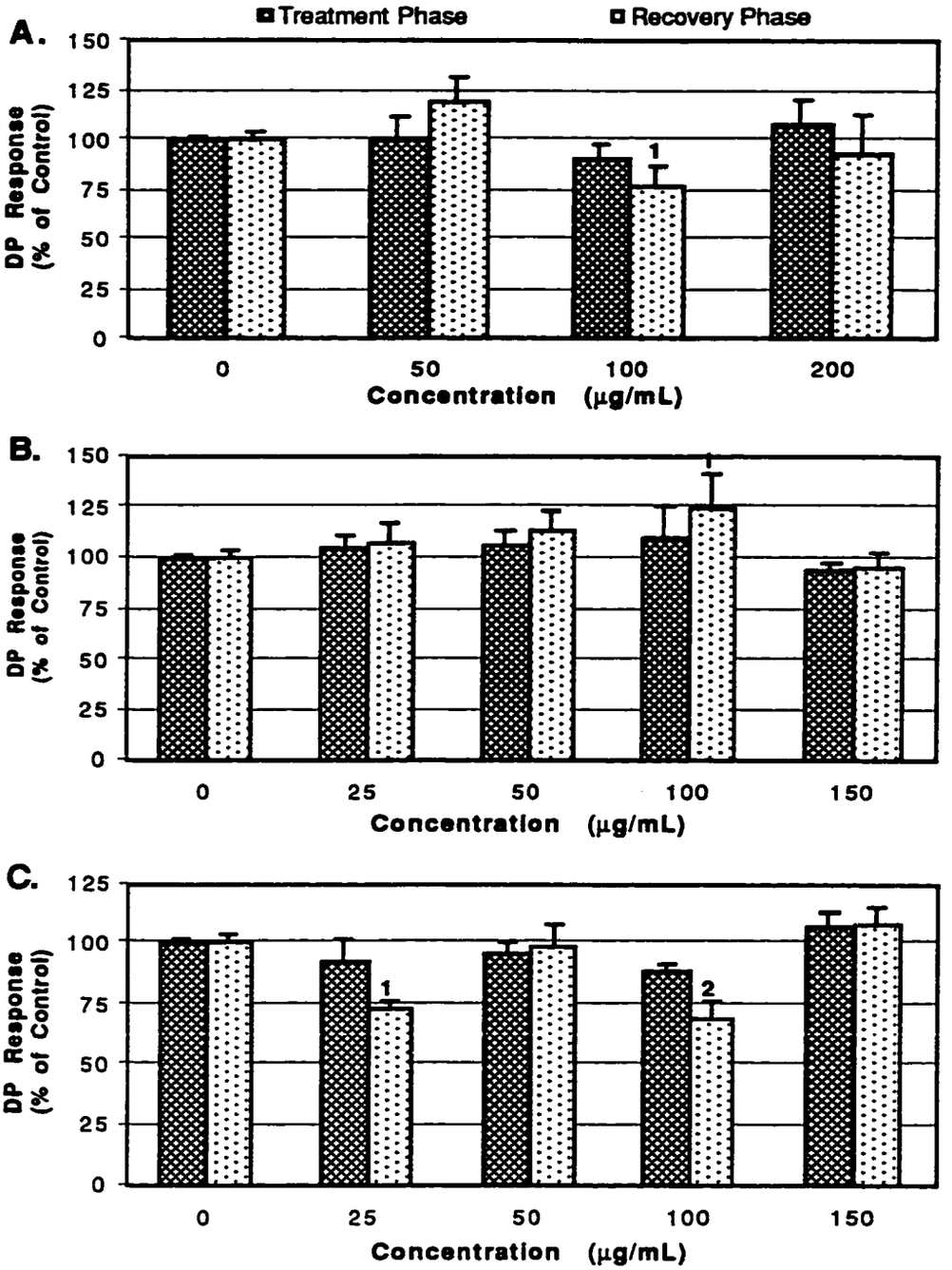


Figure 5-3. The effects of (A) chloral hydrate (0, 50, 100, 200 µg/ml), (B) trichloroethanol (0, 25, 50, 100, 150 µg/ml) and (C) trichloroacetic acid (0, 25, 50, 100, 150 µg/ml) on left ventricular developed pressure (DP Response) in isolated, perfused neonatal rabbit hearts. (Results are expressed as per cent of control values for treatment and recovery phases. Error bars indicate standard error of the group means. $^{1}p<0.05$; $^{2}p<0.01$; $^{3}p<0.001$; $^{4}p<0.0001$ *versus* respective control values.)

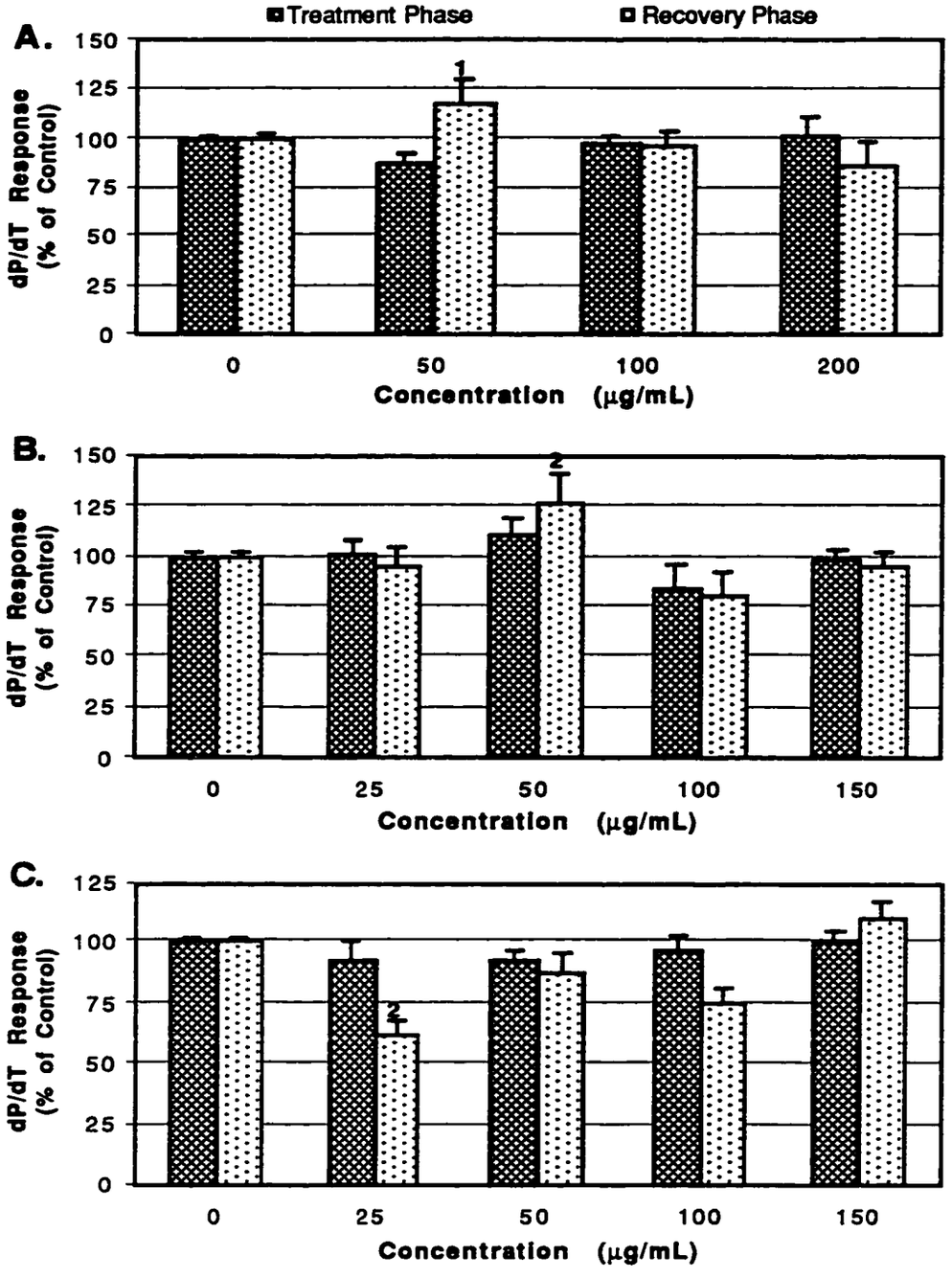


Figure 5-4. The effects of (A) chloral hydrate (0, 50, 100, 200 µg/ml), (B) trichloroethanol (0, 25, 50, 100, 150 µg/ml) and (C) trichloroacetic acid (0, 25, 50, 100, 150 µg/ml) on left ventricular contractility (dP/dT Response) in isolated, perfused neonatal rabbit hearts. (Results are expressed as per cent of control values for treatment and recovery phases. Error bars indicate standard error of the group means. $^{1}p<0.05$; $^{2}p<0.01$; $^{3}p<0.001$; $^{4}p<0.0001$ *versus* respective control values.)

5.3.2 Electrocardiographic Parameters

5.3.2.1 Heart rate

Table 5-1 shows the effects of drug treatment on heart rate (HR) for the treatment and recovery phases of the protocol. CH 100 μg/ml produced a significant decrease in HR (82% of control, $p<0.001$), which was sustained during the recovery phase (89% of control, $p<0.01$). TCE produced only a minor reduction in HR during the 100 μg/ml treatment ($p<0.05$ *vs.* control), and TCA treatment caused no significant alterations in HR.

5.3.2.2 Atrio-ventricular conduction

The P-R interval of the ECG provides a good estimate of atrio-ventricular conduction. None of the drug treatments caused any alteration in the P-R interval during treatment or recovery phases (Table 5-1).

5.3.2.3 Intra-ventricular conduction

Intra-ventricular conduction is estimated by measuring the duration of the QRS interval on the ECG. Table 5-1 shows the effects of CH, TCE and TCA on the QRS duration for treatment and recovery phases of the protocol. TCE 100 μg/ml produced a slight increase in the QRS duration (105% of control, $p<0.05$); however, this effect was not likely physiologically relevant. None of the other drug treatments produced significant changes in the QRS duration.

5.3.2.4 Cardiac arrhythmias

Figure 5-5 illustrates the effects of CH, TCE and TCA on the incidence of ectopic beats that occurred during the treatment and recovery phases of the protocol. An increase in the incidence of ectopic beats during the treatment phase was paralleled in virtually all groups by a comparable increase during the recovery phase. CH significantly increased the number of conduction defects per minute in a dose-dependent fashion in the treatment ($r^2=0.7412$; $p=0.0001$) and recovery ($r^2=0.7974$; $p=0.0002$) phases.

Table 5-1. Effects of chloral hydrate, trichloroethanol and trichloroacetic acid on electrocardiographic parameters in the isolated perfused neonatal rabbit heart.[§]

ECG Parameter		Control	Chloral hydrate (μg/ml)			Trichloroethanol (μg/ml)				Trichloroacetic acid (μg/ml)			
			50	100	200	25	50	100	150	25	50	100	150
Heart Rate	Tx	100.0	93.1	94.6	82.4[c]	96.4	95.9	89.6[a]	93.5	102.3	95.1	96.8	97.4
		[1.7]	[2.3]	[3.7]	[5.0]	[4.6]	[2.8]	[5.5]	[3.2]	[5.7]	[2.0]	[1.5]	[2.2]
	Rec	100.0	107.1	100.9	88.8[b]	98.8	102.7	94.9	101.2	96.9	92.6	97.1	99.5
		[2.8]	[4.5]	[4.2]	[4.6]	[3.9]	[4.8]	[2.5]	[3.1]	[3.3]	[4.8]	[2.5]	[1.4]
P-R interval	Tx	100.0	108.3	97.9	108.4	102.3	104.3	100.1	102.9	99.3	104.3	105.8	103.9
		[3.2]	[1.5]	[4.7]	[6.7]	[2.9]	[3.2]	[1.3]	[1.3]	[3.0]	[2.5]	[4.1]	[1.6]
	Rec	100.0	97.2	92.4	104.0	102.8	97.4	111.5	106.0	98.0	100.6	105.8	102.5
		[3.6]	[2.4]	[5.8]	[2.1]	[1.8]	[3.5]	[9.2]	[2.2]	[0.9]	[2.8]	[6.5]	[1.2]
QRS Dur'n	Tx	100.0	103.5	106.0	99.0	100.6	101.7	105.1[a]	104.6	107.1	103.5	105.5	109.1
		[1.8]	[2.7]	[1.6]	[2.0]	[3.1]	[1.7]	[2.1]	[3.3]	[3.6]	[3.8]	[3.1]	[2.5]
	Rec	100.0	104.2	108.0	100.0	99.5	105.9	110.8	107.0	106.3	108.3	102.0	106.0
		[3.1]	[2.6]	[1.6]	[3.1]	[2.8]	[3.2]	[1.7]	[2.9]	[3.5]	[2.7]	[2.8]	[3.5]

[§] Results are expressed as percent of respective control values; [S.E.]
Abbreviations: Tx=treatment phase; Rec.=recovery phase; Dur'n=duration
[a]$p<0.05$, [b]$p<0.01$, [c]$p<0.001$, [d]$p<0.0001$ *vs.* controls

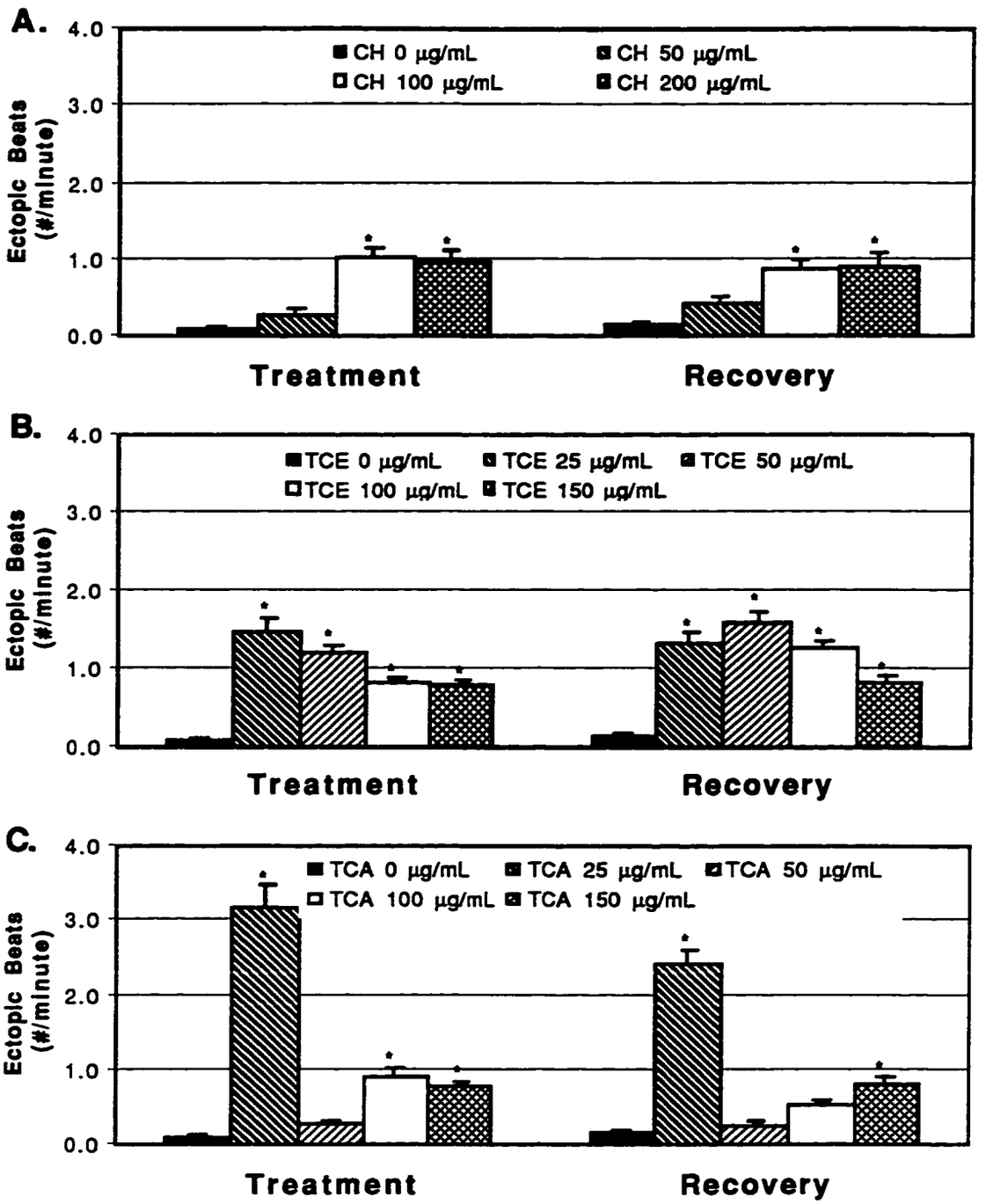


Figure 5-5. Incidence of ectopic beats in (A) chloral hydrate (0, 50, 100, 200 µg/ml), (B) trichloroethanol (0, 25, 50, 100, 150 µg/ml) and (C) trichloroacetic acid (0, 25, 50, 100, 150 µg/ml) treated isolated, perfused neonatal rabbit hearts. (Results are expressed as number of ectopic beats observed per minute in treatment and recovery phases. Error bars indicate the standard error of the group means. *$p<0.05$ *versus* the respective control values.)

While no clear dose-response relationship can be detected for TCE and TCA treatments at the concentrations tested, it is apparent that these compounds significantly increased the number of conduction disturbances per minute in both treatment and recovery phases ($p<0.05$ *vs.* controls). It is possible that a dose-response relationship between TCE or TCA treatment and the incidence of ectopic beats exists for concentrations less than 25 μg/ml. CH 100 and 200 μg/ml caused significant increases in the incidence of conduction defects during both the treatment and recovery phases ($p<0.05$ *vs.* controls). All concentrations of TCE tested produced significantly more conduction defects than did the control protocol ($p<0.05$ *vs.* controls). TCA 25 μg/ml caused a large increase in the number of conduction defects observed in both treatment and recovery phases ($p<0.05$ *vs.* controls). TCA 100 and 150 μg/ml treatments produced more moderate increases in conduction defects ($p<0.05$ *vs.* controls).

Table 5-2 summarizes the various ECG abnormalities detected for each of the treatment groups. Control hearts were found to be relatively free of rhythm disturbances, with the only exceptions being very infrequent premature atrial and ventricular contractions (PAC's and PVC's). Generally, atrio-ventricular (AV) conduction blocks were not observed in CH and TCA groups. However, TCE (50 and 100 μg/ml) caused Mobitz type I second-degree AV block. In one TCE treatment group (100 μg/ml) evidence of an ectopic pacemaker was found. Bundle branch blocks were rarely encountered in CH, TCE and TCE treated hearts. Premature contractions occurred quite frequently in all treatment groups; however, PVC's were especially evident in CH and TCE treated hearts. Atrial and ventricular bigeminy were observed only in CH and TCE groups, although the occurrences were relatively infrequent. In all instances of premature beats (PAC/atrial bigeminy, PVC/ventricular bigeminy), the episodes were of a limited nature. Paroxysmal supra-ventricular tachycardia occurred occasionally in TCE and TCA treated hearts. Brady-tachy syndrome (a manifestation of the sick sinus syndrome) was observed only with TCA 150 μg/ml treatment.

Table 5-2. Cardiac arrhythmias occurring in the isolated, perfused neonatal rabbit heart during control, chloral hydrate, trichloroethanol or trichloroacetic acid treatment.

Cardiac Arrhythmias	Control	Chloral Hydrate (μg/mL)			Trichloroethanol (μg/mL)				Trichloroacetic Acid (μg/mL)			
		50	100	200	25	50	100	150	25	50	100	150
Premature ventricular contraction	◊	◊	◊◊	◊◊	◊◊	◊◊	◊◊	◊◊	◊◊		◊	◊
Ventricular bigeminy			◊	◊	◊	◊	◊	◊	◊			
Ventricular tachycardia			◊	◊	◊	◊	◊		◊			◊
Ventricular fibrillation												◊
Premature atrial contraction	◊	◊	◊	◊◊	◊	◊	◊	◊	◊	◊	◊	◊
Atrial bigeminy			◊				◊		◊			
Premature supra-ventricular tachycardia						◊	◊	◊	◊		◊	
Sick sinus syndrome												◊
2° Atrioventricular block						◊	◊					
Ectopic pacemaker							◊					
Bundle branch block		◊						◊	◊			

◊ : denotes occurrence in at least one experiment
◊◊: denotes occurrence in at least four experiments

Electrocardiographic tracings illustrating specific examples of cardiac arrhythmias observed in neonatal rabbit hearts are located in Figures C-1 to C-5 (Appendix C).

5.4 DISCUSSION AND CONCLUSIONS

CH-related arrhythmias in the pediatric population have been documented (Anyebuno and Rosenfeld, 1991; Hirsch and Zauder, 1986; Nordenberg *et al.*, 1971; Silver and Stier, 1971). No effort has been made, however, to determine the relative susceptibility of neonates to this toxic effect of CH. Determination of the relative cardiac toxicity in this vulnerable population is especially important in light of the accumulation of TCE and TCA in immature patients, especially after multiple dosing (Hindmarsh *et al.*, 1991; Mayers *et al.*, 1991).

Previous investigations in this laboratory have demonstrated that CH, TCE and TCA have general cardiac depressant effects in the isolated, perfused adult rabbit heart at concentrations routinely encountered in clinical situations (Fandrey *et al.*, 1992). More importantly, TCA produced severe and persistent conduction disturbances, resulting ultimately in ventricular tachycardia and/or ventricular fibrillation at all concentrations tested in this model system.

The present investigation was undertaken to determine whether the cardiac toxicity demonstrated in the adult heart model would be observed in the neonatal rabbit heart. Species variation in the magnitude of response to pharmacologic agents is quite common; however, the therapeutic plasma concentration of a particular agent observed in animals is usually comparable to that seen in humans (Baggot, 1992). For this reason, concentrations of CH, TCE and TCA selected for this investigation were based on the range of plasma concentrations observed in human neonatal pharmacokinetic studies (Hindmarsh *et al.*, 1991; Mayers, 1992). The perfused heart preparation is a valuable tool for the evaluation of interventions that, in the intact organism, may have direct and/or indirect actions on the heart (Henry *et al.*, 1977). Since

the rabbit heart has been widely employed for studies of cardiac function and development (Roberts, 1991), and since drugs tend to induce similar qualitative effects in different species (Weiner and Newberne, 1977), this model system should provide valuable information regarding the potential for direct cardiotoxic effects of CH, TCE and TCA in the developing heart.

The patency of the coronary vasculature was not detrimentally affected by the experimental protocol itself, as evidenced by the maintenance of CF throughout the control protocols (Appendix C, Table C-1). Only CH (50 µg/ml) and TCA (100µg/ml) produced modest reductions in CF. The fact that the alterations produced by CH and TCA on CF were both relatively minor and also reversible suggests that this effect was not likely instrumental in any observed cardiac toxicity.

CH 50 µg/ml produced a slight inhibition of myocardial oxygen consumption in the treatment phase of the protocol, while the corresponding recovery phase for this group demonstrated a significant increase in MVO_2. The response in the recovery phase most likely represents a rebound effect following a period of relative tissue hypoxia produced by the decreased CF (ischemia) during the treatment phase. This phenomenon was not observed in any other treatment group. TCE proved to be devoid of any effect on the MVO_2. TCA (100 µg/ml), however, produced a reduction in MVO_2 that was progressive throughout both the treatment and recovery phases.

Ventricular performance was assessed by estimating the left ventricular compliance (LVDP) and contractility (dP/dT). The rate of rise of left ventricular pressure is affected by preload, afterload, contractility and HR (Prasad *et al.*, 1991). However, this parameter constitutes a reasonable index of myocardial contractility in the isolated heart system since preload and afterload are fixed (Berne and Levy, 1993a; Henry *et al.*, 1977; Riggs *et al.*, 1986). Both compliance and contractility were maintained throughout the treatment phases for CH, TCE and TCA. All treatments, however, produced alterations in ventricular performance that were manifested in their respective recovery phases. These effects, however, were unpredictable and did not appear to be

dose-related. In fact, the highest concentrations tested for each of the compounds failed to produce any change in ventricular performance. CH (100 µg/ml) and TCA (25 and 100 µg/ml) reduced LVDP (increased compliance) in the recovery phase. In the case of TCA treatment, this alteration was paralleled by reduced dP/dT in the same treatment groups. These alterations, along with the previously mentioned reduction in MVO_2, would appear to reflect a progressive deterioration of tissue viability. The degree of ischemia produced by the TCA treatments does not appear to be sufficient to account for the progressive deterioration of these parameters. It is possible that these effects are mediated through a direct action of TCA on the coronary vascular endothelium. TCA has a well documented corrosive nature (Windholz, 1983). Disruption of the coronary endothelium may prevent the transfer of oxygen from the buffer solution to the myocardium in the absence of frank ischemia. Tissue hypoxia would be predicted to adversely affect ventricular performance. Both CH (50 µg/ml) and TCE (50 µg/ml) displayed positive inotropism in their recovery phases. This could indicate a rebound response to reversible myocardial damage incurred during the drug treatments.

Isolated neonatal rabbit hearts proved to be remarkably resistant to alterations of myocardial rhythm and conduction. The highest concentration of CH (200 µg/ml) produced moderate bradycardia, which was sustained through the recovery phase. The most likely cause of the reduced heart rate would be sinus bradycardia, since atrio-ventricular blocks and atrio-ventricular junctional escape rhythms were not observed. TCE (100 µg/ml) produced a very slight reduction in HR in the treatment phase only. TCA had no effect on heart rate at any concentration tested. None of the compounds produced any attenuation of the atrio-ventricular conduction velocity, as estimated by the P-R interval. A very slight increase in QRS duration, representing intra-ventricular conduction, was observed in one TCE (100 µg/ml) group. None of the other interventions produced any delay in intra-ventricular conduction.

While CH, TCE and TCA produced no notable alterations in the major myocardial conduction pathways, a number of conduction defects (primarily

representing atrial and ventricular ectopic beats) were observed in the treated hearts. The conduction disturbances elicited by CH and its metabolites consisted largely of premature contractions, of both atrial (PAC/atrial bigeminy) and ventricular (PVC/ventricular bigeminy) origin. The incidence of atrial premature beats was approximately equal for each of the three compounds investigated. PVC's, however, appeared to be more prevalent in CH and TCE treated hearts. Generally, atrio-ventricular blocks were not seen, with the only exception being type I second-degree AV block that occurred in TCE (50 and 100 μg/ml) treated hearts. Bundle branch blocks were observed rarely in the treated hearts. Brady-tachy syndrome is a term used to describe a subset of the sick sinus syndrome in which tachyarrhythmias occur in an alternating fashion with bradyarrhythmias (Goldberger and Goldberger, 1990b). This syndrome of alternating bradycardia and tachycardia was observed only with TCA treatment. Paroxysmal supraventricular tachycardia was occasionally present in TCE and TCA treated hearts, while ventricular tachycardia occurred in isolated bursts with all drug interventions. Ventricular fibrillation, which was considered the endpoint of arrhythmia, occurred only with the highest concentration of TCA. No clear pattern of rhythm and conduction disturbances is evident; however, TCE appeared to cause the broadest range of dysrhythmias. It is of note that all conduction disturbances occurred as isolated events.

With the exception of conduction defects, all results have been expressed as time-averaged values encompassing the treatment and recovery phases, respectively. This approach was used to enable the assessment of the differences of the physiological parameters from their control values over time. The cardiovascular effects of anesthetic agents are known to be time-dependent; thus, measurements observed at a single time point can be misleading in the study of such effects. Therefore, the investigation of anesthetic agents may be incomplete without including the effects of time in the overall assessment of toxicity (Eger *et al.*, 1970). Since there is evidence that CH and its active metabolite TCE exert their cardiotoxic effects in a fashion

analogous to halothane (Brown and Cade, 1980), then the effects exerted over time would be of importance in their toxicity assessment. Use of time-averaged data for treatment and recovery phases also allowed for the assessment of the overall effects of the drug/metabolite, minimizing the impact of normal physiological variation over time on the interpretation of the results. This approach should provide more relevant information than the assessment of various effects for each ten minute interval (Urquhart, 1981).

CH and its metabolites appear to be general cardiac depressants with arrhythmogenic potential in an adult rabbit heart model (Fandrey *et al.*, 1992). However, these same compounds produce only minimal cardiotoxic effects in neonatal rabbit hearts. Resistance to cardiac toxicity in fetal and neonatal mammals is not without precedent. The resistance of the neonatal myocardium to deleterious effects of ischemia and subsequent reperfusion is well-established (Grice *et al.*, 1987; McCully *et al.*, 1994; Murashita *et al.*, 1992). Fetal and neonatal hearts have also been shown to be resistant to the cardiotoxic effects of β-adrenoceptor agonists (analogs of isoproterenol) used in tocolytic therapy. It has been postulated that the latter effect is due to immature sympathetic innervation in the perinatal heart (Kast and Hermer, 1993). The response of the isolated neonatal rabbit heart to CH and its metabolites apparently reflects another situation in which physiological immaturity serves to protect the target organ from drug toxicity.

6. A COMPARATIVE STUDY OF THE DISPOSITION AND ACCUMULATION OF CHLORAL HYDRATE AND ITS METABOLITES IN ISOLATED PERFUSED ADULT AND NEONATAL RABBIT HEARTS

6.1 INTRODUCTION

Chloral hydrate is a commonly used sedative in pediatric and geriatric patients (Cook *et al.*, 1992; Hoskins, 1984; Marx *et al.*, 1993). Throughout its extremely long history of use, a number of serious toxic effects have been attributed to CH ingestion (Ganepola, 1992; Graham *et al.*, 1988; Hoskins, 1984; Steinberg, 1993). In particular, CH use has been plagued by reports of cardiac arrhythmias, especially in situations of over-dose, in both children and adults (Graham *et al.*, 1988; Gustafson *et al.*, 1977; Hirsch and Zauder, 1986; Marshall, 1977). Few investigations of the direct effects of CH and/or its metabolites (TCE and TCA) on the heart have been reported (Gross and Hoff, 1968; Riggs *et al.*, 1986; Trulson and Ulissey, 1987; White and Carlson, 1981). Previous investigations suggest that CH and its phase I metabolites trichloroethanol and trichloroacetic acid are myocardial depressants, and produce conduction defects which may lead to cardiac arrhythmias in a rabbit heart model (Fandrey *et al.*, 1992).

Chloral hydrate is rapidly metabolized in adult subjects both by reduction to trichloroethanol and by oxidation to trichloroacetic acid. TCE is further conjugated with glucuronic acid prior to excretion in the urine (Breimer *et al.*, 1974; Sellers *et al.*, 1978). When administered to healthy adult volunteers, CH is nearly undetectable within minutes of administration (Breimer *et al.*, 1974; Gorecki *et al.*, 1990). However, metabolism of CH in the neonate is characterized by immaturity of hepatic and renal elimination processes. Contrary to the situation in the adult, CH was detectable for several hours after a single oral dose (50 mg/kg) in neonates and young

children (Mayers *et al.*, 1991). The elimination half-lives of both TCE (30-40 h) (Gershanik *et al.*, 1981; Hindmarsh *et al.*, 1991; Mayers *et al.*, 1991) and TCA (>6 days) (Hindmarsh *et al.*, 1991; Mayers *et al.*, 1991) are substantially prolonged in neonates when compared to adult volunteers (8-12 h and 67-100 h, respectively) (Breimer *et al.*, 1974; Sellers *et al.*, 1972b; Sellers *et al.*, 1978). Of particular concern in the neonatal population is the persistence of TCA, a potentially toxic metabolite, even after a single oral dose (Hindmarsh *et al.*, 1991; Mayers *et al.*, 1991).

The present investigation utilized isolated, perfused adult and neonatal rabbit heart models to determine whether sequestration of CH and/or one of its metabolites occurs, and to estimate the non-compartmental pharmacokinetic parameters associated with their myocardial disposition. Since drug-induced toxicity is frequently related to the accumulation of an agent in a particular organ, the elucidation of tissue accumulation kinetics in the target organ should provide important insights in determining the pathogenesis of CH-induced toxicity in adult and neonatal hearts.

6.2 EXPERIMENTAL

6.2.1 Materials

6.2.1.1 Chemicals

Chloral hydrate was supplied by Bristol-Myers Squibb Canada, Inc. (Montreal, PQ). Trichloroacetic acid was obtained from Fluka Chemicals, Terochem Laboratories Ltd. (Edmonton, AB). Both CH and TCA were dried overnight under vacuum prior to use. Trichloroethanol, methyl trichloroacetic acid (MTCA) and the internal standard, 1,2,3-trichloropropane (TCP), were purchased from Aldrich Chemical Co. (Milwaukee, WI). N-methyl-N-nitroso-*p*-toluene sulfonamide (Diazald) was obtained from Sigma Chemical Co. (St. Louis, MO). Analytical grade diethyl ether was purchased from Fisher Scientific (Fair Lawn, NJ), and was freshly distilled prior to use. All other

reagents and solvents were purchased from either Sigma Chemical Co. (St. Louis, MO) or BDH Chemicals (Toronto, ON), and were of analytical grade.

6.2.1.2 Animals

New Zealand neonatal (7-10 day) and adult white rabbits (2-3 kg) of either sex were obtained from the licensed breeding facility at the Animal Resources Centre, University of Saskatchewan (see Appendix A, Tables A-1 and A-2). Neonatal rabbits were obtained on the morning of the experiment, and were housed in a warm (30-35°C), dark environment prior to use. All adult rabbits were housed in stainless-steel cages with access to standard rabbit laboratory chow and tap water *ad libitum*, in accordance with the recommendations of the Canadian Council on Animal Care (CCAC).

6.2.2 Methods

6.2.2.1 Heart Perfusion

Adult rabbits were sacrificed by cervical dislocation and neonatal rabbits were sacrificed by decapitation according to accepted CCAC protocols. Anesthetic exposure was avoided to prevent any potential for interaction with the subsequent drug (CH, TCE, or TCA) treatments. The hearts were rapidly excised and immediately placed in ice-cold perfusion buffer. The aorta was cannulated as quickly as possible (within 1-2 minutes) and Langendorff perfusion (Langendorff, 1895) was initiated at a high flow rate to clean the coronary arteries. Once the arteries were sufficiently flushed, the heart was perfused as described previously (Prasad *et al.*, 1992b). Retrograde perfusion was maintained at a constant pressure of 100 cmH_2O with a bicarbonate buffer, pH 7.4, of the following composition (in mM): sodium chloride, 154.0; potassium chloride, 5.63; sodium bicarbonate, 5.95; calcium chloride, 2.16; and glucose, 5.55. The buffer passed through an in-line filter prior to heart perfusion, and was equilibrated with a gas mixture of 95% O_2 and 5% CO_2 and maintained at 37°C throughout the experimental protocol. The rates of perfusion were 8.4 ± 0.4 and 11.6 ± 0.6 mL/minute for neonatal and adult

hearts, respectively. All heart preparations were allowed to stabilize for at least 45 minutes prior to any interventions.

6.2.2.2 Drug Administration

Drug solutions (CH 50, 100, and 200 μg/ml; TCE and TCA 25, 50, 100, 150 μg/ml) were prepared fresh daily using the buffer as solvent. The perfusion apparatus was connected such that separate reservoirs for pure buffer and drug/buffer solutions could be employed. Drug administration via the buffer solution was initiated after stabilization of the heart and continued for 60 minutes (treatment phase). Following the treatment phase, the perfusion was switched back to pure buffer and the heart was monitored for an additional 60 minutes (recovery phase). Ten control experiments were performed, while the drug interventions were repeated 6-7 times for each dosing level. Perfusate samples (1 ml) were collected every 10 minutes throughout the experimental protocol, and were subsequently stored at -70°C with appropriate standard and quality control samples until analysis.

6.2.2.3 Gas Chromatographic Analysis

Analysis of CH and its metabolites (TCE and TCA) was carried out as described previously (Gorecki *et al.*, 1990). Briefly, aliquots (50 μl) of buffer (either standards or samples) and internal standard (TCP; 500 μg/ml) were treated with sulfuric acid (250 μl; 3 M) and subsequently extracted into freshly distilled diethyl ether (5.0 ml). An aliquot (2 ml) of the ether layer was derivitized with ethereal diazomethane to facilitate the methylation of the TCA metabolite (MTCA). Aliquots (1 μl) of the resulting mixture were injected into the gas chromatograph. Representative chromatograms for the determination of CH, MTCA and TCE are found in Appendix D (Figures D-1 and D-2).

Gas chromatographic analysis was carried out on a Varian Model 3700 gas chromatograph (Varian Assoc., Palo Alto, CA) equipped with a ^{63}Ni electron-capture detector and a coiled-glass column (2.44 m x 2 mm i.d.) packed with Supelco GP 10% SP-1200/1% H_3PO_4 on 80-100 mesh

Chromosorb W AW (Supelco, Bellefonte, PA). Isothermal analysis was carried out with injection port, column oven and detector temperatures of 160, 115 and 300°C, respectively. Argon-methane (95:5) was used as the carrier gas at a flow-rate of 12.0 ml/min. Peak-height determination and quantitation was obtained with the use of a Shimadzu Model C-R3A Chromatopac integrator-recorder (Shimadzu, Kyoto, Japan).

Analytical recoveries of CH, MTCA and TCE from aqueous buffer were determined by comparison of the peak height ratios of standard to internal standard obtained from extracted spiked buffer to those obtained from direct injection of standards containing equivalent amounts of the analytes. The recoveries expressed as percent (S.E.M.) for CH, MTCA and TCE were 108.1 (6.3), 99.2 (5.3) and 85.6 (5.2) respectively. Recovery data for CH, MTCA and TCE are summarized in Figure D-4 (Appendix D). Interday variation of peak height ratios was less than 10% for individual sample/standard injections. Intraday variability and stability data are illustrated graphically in Figure D-3 (Appendix D).

Calibration curves in the range of 0.1-200 μg/ml (CH) and 0.1-150 μg/ml (TCE and MTCA) were generated using perfusion buffer as the solvent. These ranges were extended beyond those previously reported, and resulted in nonlinear ECD responses for CH and TCE analytes. The calibration curves (constructed by plotting the ratios of peak height of the respective analyte to that of the internal standard against the analyte concentration) for CH and TCE were thus fitted using a logarithmic-logarithmic ("power") curve-fitting procedure ($\ln y = \ln B + M \ln x$; Hewlett Packard 27S Scientific calculator). The MTCA calibration curve was adequately described by a linear regression procedure. Correlation coefficients for calibration curves were 0.999, 0.999 and 0.998 for CH, TCE and MTCA respectively (Appendix D, Figures D-5 and D-6).

6.2.2.4 Pharmacokinetic Analysis

Myocardial pharmacokinetic parameters were determined using standard non-compartmental techniques (Gibaldi and Perrier, 1982; Gillespie, 1991b). Area under the buffer concentration-time curve (AUC; μg/ml x min) and its first moment (AUMC; μg/ml x min^2) were determined by Lagrange interpolation (Rocci and Jusko, 1983). These parameters were used to calculate the mean residence times (MRTs; min) for the drug infusions (where T is time of drug infusion in minutes) according the following relationship (Gibaldi and Perrier, 1982):

$$MRT = (AUMC / AUC) - T/2 \qquad (6\text{-}1)$$

Clearance (CL; ml/min) was determined from the input rate (k_0; ml/min) and concentration at steady-state (C_{SS}; μg/ml) (Gillespie, 1991b):

$$CL = k_0 / C_{SS} \qquad (6\text{-}2)$$

The apparent volume of distribution at steady-state (V_{SS}; ml) was calculated using k_0, time of drug infusion (T; min), AUC and AUMC (Gibaldi and Perrier, 1982):

$$V_{SS} = \frac{k_0 T \cdot AUMC}{AUC^2} - \frac{k_0 T^2}{2\,AUC} \qquad (6\text{-}3)$$

The amount of drug sequestered in the heart at steady-state (A_{SS}) was calculated using the following relationship (Gillespie, 1991b):

$$A_{SS} = V_{SS} \times C_{SS} \qquad (6\text{-}4)$$

6.2.2.5 Statistical Analysis

Results were analysed by one- (within each age group) or two-way (to test treatment and age effects) analysis of variance (SuperANOVA, Abacus Concepts Inc., 1989). If the analysis of variance demonstrated significant results, appropriate *post hoc* tests (Bonferroni/Dunn all means multiple comparison procedure) were used to identify significant treatments (Ludbrook, 1991). Correlation between variables was determined by the standard least-squares regression technique. A $p < 0.05$ was considered to be statistically significant.

6.3 RESULTS

Figures 6-1 and 6-2 represent the mean log concentration-time profiles for various concentrations of CH (A), TCE (B) and TCA (C) in adult and neonatal rabbit hearts, respectively. Steady-state was established within 20-30 minutes of initiation of drug administration in all treatments. Following cessation of drug administration, drug concentrations in the perfusate solution declined in a bi-exponential fashion for all groups, with the exception of TCE 150 µg/ml adult group (see Figure 6-1B), in which elimination appeared to be mono-exponential.

The mean log concentration-time plots were used to estimate various non-compartmental pharmacokinetic parameters (Tables 6-1 and 6-2). Two-way ANOVA demonstrated that the neonatal hearts achieved significantly higher concentrations at steady-state (C_{SS}) than the corresponding adult heart groups ($p<0.0001$). C_{SS} increased in a linear fashion with respect to dose in all treatment groups ($r = 0.9960$-0.9995; $p<0.01$); however, CH and TCA in the adult hearts displayed disproportionately increased C_{SS} at higher perfusate concentrations. The amount of drug in the hearts at steady-state (A_{SS}) also displayed disproportionate increases at higher doses of CH and TCE in the adult hearts. In the case of CH, this increase was exponential in nature (Figure 6-3A). Accumulation of CH and TCA in the neonatal hearts was linear

and directly proportional to administered dose; however, accumulation of TCE appeared to approach a plateau with increasing dose (Figure 6-3B).

One-way ANOVA of apparent volume of distribution at steady-state (V_{SS}) showed that this parameter was not significantly different between treatments within the adult and neonatal groups, respectively (Tables 6-1 and 6-2). However, the average V_{SS} of the adult hearts was significantly greater than that of the neonatal hearts ($p<0.0001$).

Mean residence times (MRTs) were equivalent for CH, TCE and TCA within the adult (Table 6-1) and neonatal (Table 6-2) groups. The MRTs in the adult hearts, however, were significantly less than those observed in neonatal hearts ($p<0.0001$). Clearance values for CH, TCE and TCA were significantly enhanced in adult *vs.* neonatal hearts ($p<0.0001$). While CH and TCA clearance in both adult and neonatal hearts demonstrated a slight tendency towards nonlinearity at higher doses, assessment of the slopes of the plots of clearance *vs.* dose revealed that none of the slopes was significantly different from zero

Figure 6-4

Tables 6-3 and 6-4 describe the relevant pharmacokinetic parameters for metabolite formation after CH, TCE and TCA administration in adult and neonatal hearts, respectively. Metabolism in individual hearts was highly variable and unpredictable; therefore, no attempt has been made to assess mass-balance for any of the treatments. It is evident, however, that the heart is a site of metabolism for all three compounds.

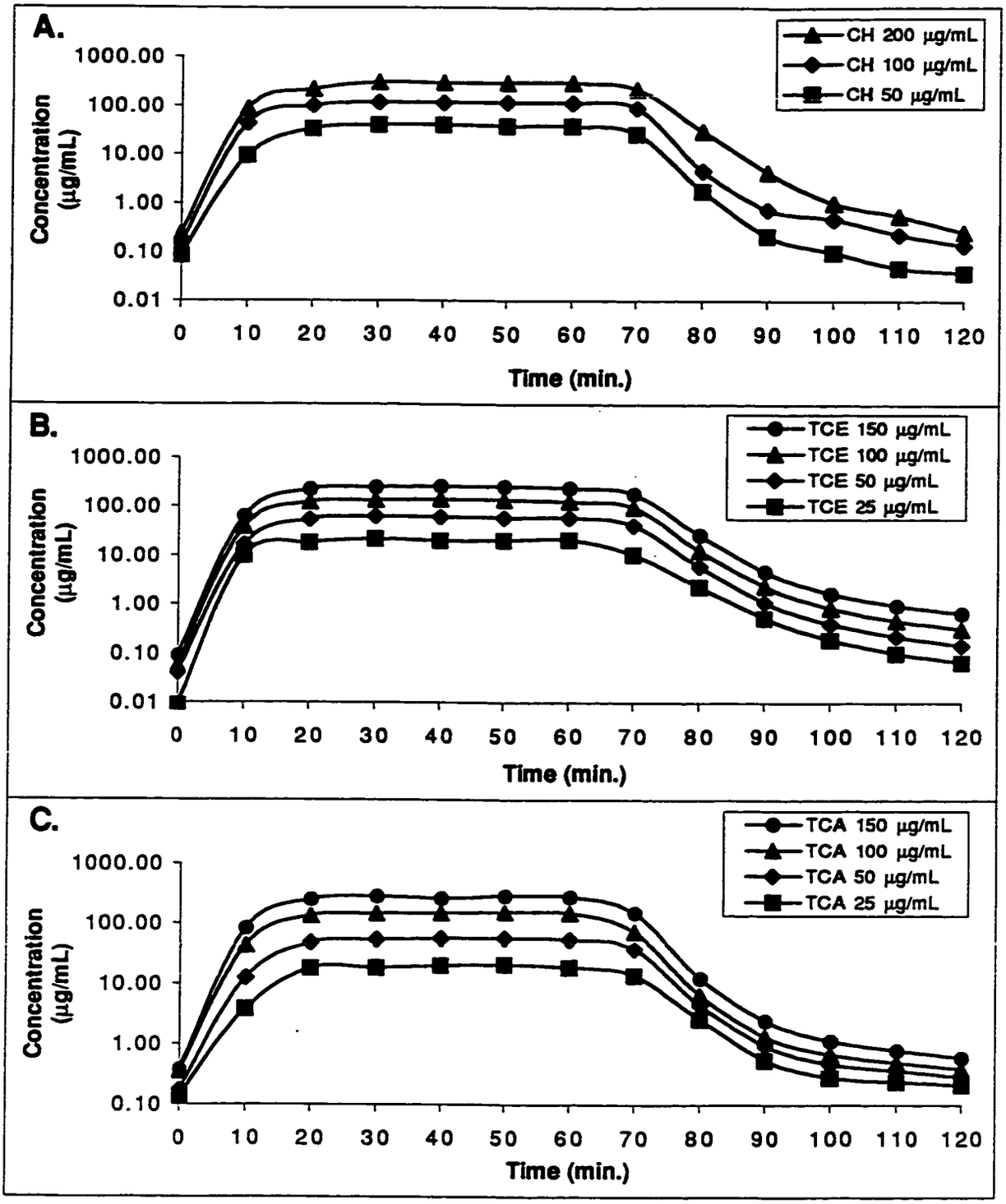


Figure 6-1. The mean log concentration-time profiles for (A) chloral hydrate (50, 100 and 200 µg/ml), (B) trichloroethanol (25, 50, 100 and 150 µg/ml) and (C) trichloroacetic acid (25, 50, 100 and 150 µg/ml) in isolated, perfused adult rabbit hearts. Each point represents the average of 4-5 experiments.

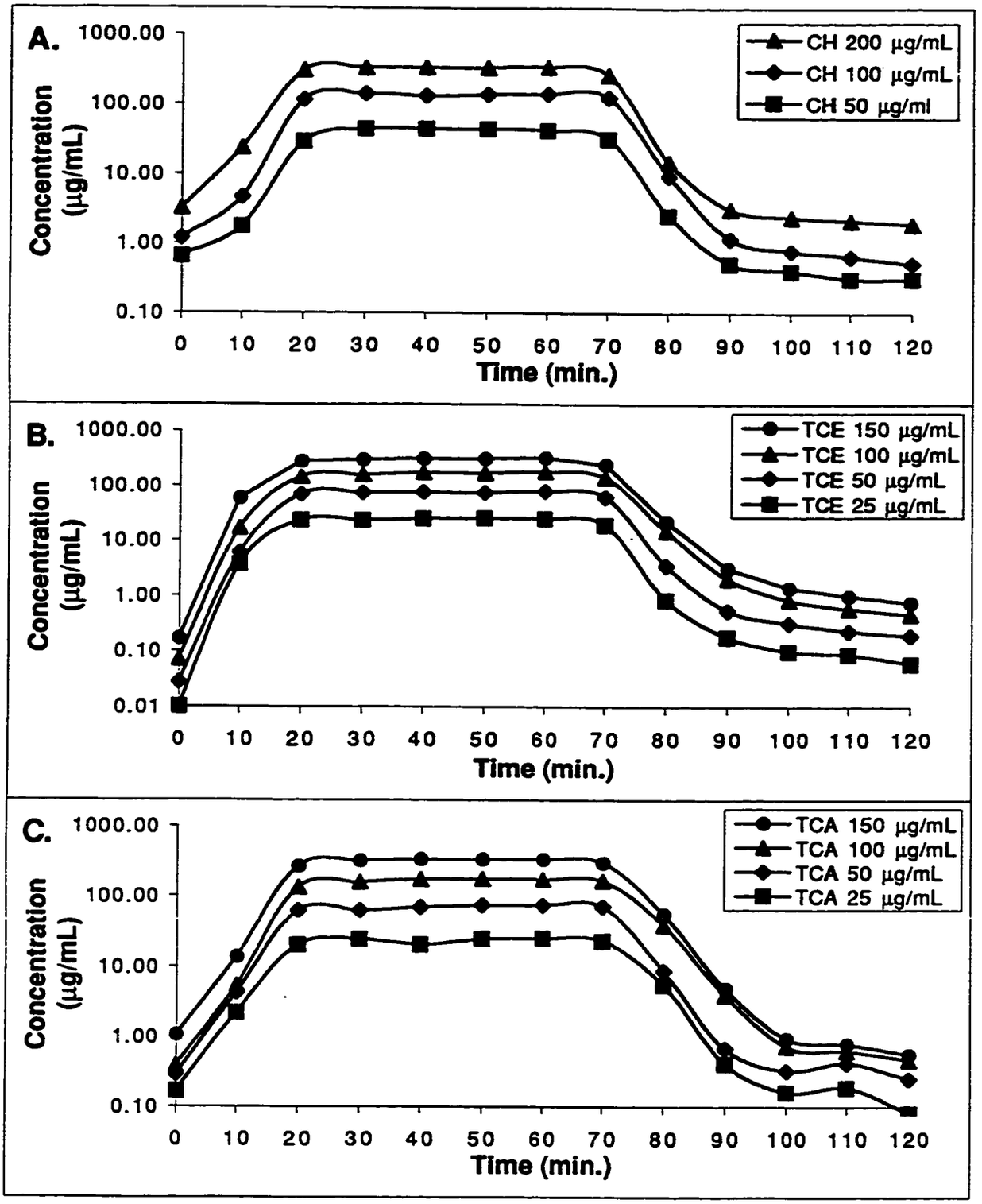


Figure 6-2. The mean log concentration-time profiles for (A) chloral hydrate (50, 100 and 200 µg/ml), (B) trichloroethanol (25, 50, 100 and 150 µg/ml) and (c) trichloroacetic acid (25, 50, 100 and 150 µg/ml) in isolated, perfused neonatal rabbit hearts. Each point represents the average of 4-5 experiments.

Table 6-1. Non-compartmental pharmacokinetic parameters determined for chloral hydrate, trichloroethanol and trichloroacetic acid in isolated, perfused adult rabbit hearts. Values represent the means of 4-5 replicates. [§]

	Chloral hydrate (μg/ml)			Trichloroethanol (μg/ml)				Trichloroacetic acid (μg/ml)			
	50	100	200	25	50	100	150	25	50	100	150
k_0 (μg/min)	580	1160	2320	290	580	1160	1740	290	580	1160	1740
C_{ss} (μg/ml)	38.40	75.12	176.17	20.52	39.65	72.98	117.74	19.38	35.71	92.67	127.26
AUC	2243.38	4664.33	10244.15	1243.12	2359.80	4441.80	7045.93	1194.40	2080.98	5250.45	7521.58
AUMC	96176.23	197892.58	457553.15	51641.20	104190.18	191848.63	311879.25	53680.13	90137.48	214460.25	317315.30
MRT (min)	12.87	12.43	14.66	11.54	14.15	13.19	14.26	14.94	13.32	10.85	12.19
CL (ml/min)	15.10	15.44	13.17	14.13	14.63	15.89	14.78	14.96	16.24	12.52	13.67
V_{ss} (ml)	199.65	185.44	199.27	161.56	208.70	206.70	211.35	217.69	222.66	143.77	169.16
A_{ss} (μmol)	6.21	11.29	28.45	2.97	7.42	13.53	22.32	3.46	6.52	10.93	17.66

[§] **Abbreviations:** k_0 (input rate); C_{ss} (concentration at steady-state); AUC (area-under-the-curve); AUMC (area-under-the-moment-curve); MRT (mean transit time); CL (clearance); V_{ss} (apparent volume of distribution at steady-state); A_{ss} (amount of drug accumulated at steady-state).

Table 6-2. Non-compartmental pharmacokinetic parameters determined for chloral hydrate, trichloroethanol and trichloroacetic acid in isolated, perfused neonatal rabbit hearts. Values represent the means of 4-5 replicates.[§]

	Chloral hydrate (μg/ml)			Trichloroethanol (μg/ml)				Trichloroacetic acid (μg/ml)			
	50	100	200	25	50	100	150	25	50	100	150
k_o (μg/min)	420	840	1680	210	420	840	1260	210	420	840	1260
C_{ss} (μg/ml)	43.75	93.75	198.82	24.45	51.08	94.08	139.70	23.40	48.55	99.69	163.69
AUC	2419.9	5614.725	11646.46	1445.875	2972.725	5528.25	8253.65	1448.125	2736.975	5900.85	9429.8
AUMC	111947.8	258652.7	541307.2	63685.03	134236.9	254460.2	359840.3	68101.1	127983.1	285015.1	430534.4
MRT (min)	16.26	16.07	16.48	14.05	15.16	16.03	13.60	17.03	16.76	18.30	15.66
CL (ml/min)	9.60	8.96	8.45	8.59	8.22	8.93	9.02	8.97	8.65	8.43	7.70
V_{ss} (ml)	169.34	144.22	142.62	122.39	128.48	146.13	124.55	148.16	154.32	156.30	125.52
A_{ss} (μmol)	48.68	88.84	186.32	21.77	47.75	100.01	126.57	23.06	49.84	103.65	136.68

[§] **Abbreviations:** k_o (input rate); C_{ss} (concentration at steady-state); AUC (area-under-the-curve); AUMC (area-under-the-moment-curve); MRT (mean transit time); CL (clearance); V_{ss} (apparent volume of distribution at steady-state); A_{ss} (amount of drug accumulated at steady-state).

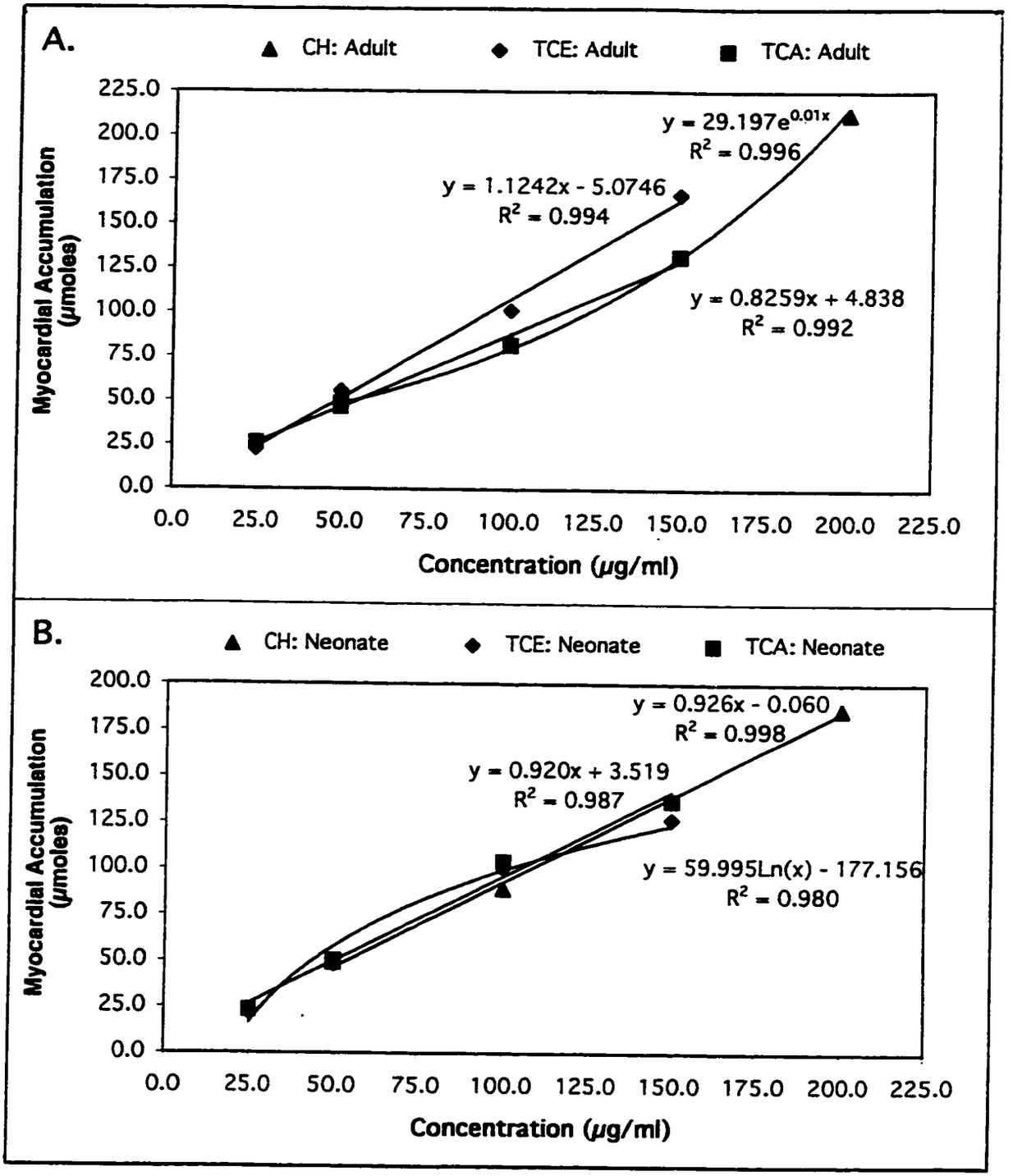


Figure 6-3. The amounts of chloral hydrate (50, 100 and 200 µg/ml), trichloroethanol (25, 50, 100 and 150 µg/ml) and trichloroacetic acid (25, 50, 100 and 150 µg/ml) accumulated at steady-state in isolated, perfused adult (A) and neonatal (B) rabbit hearts. Each point represents the average of 4-5 experiments. Curves were fitted by standard regression techniques, and are displayed with their respective regression coefficients.

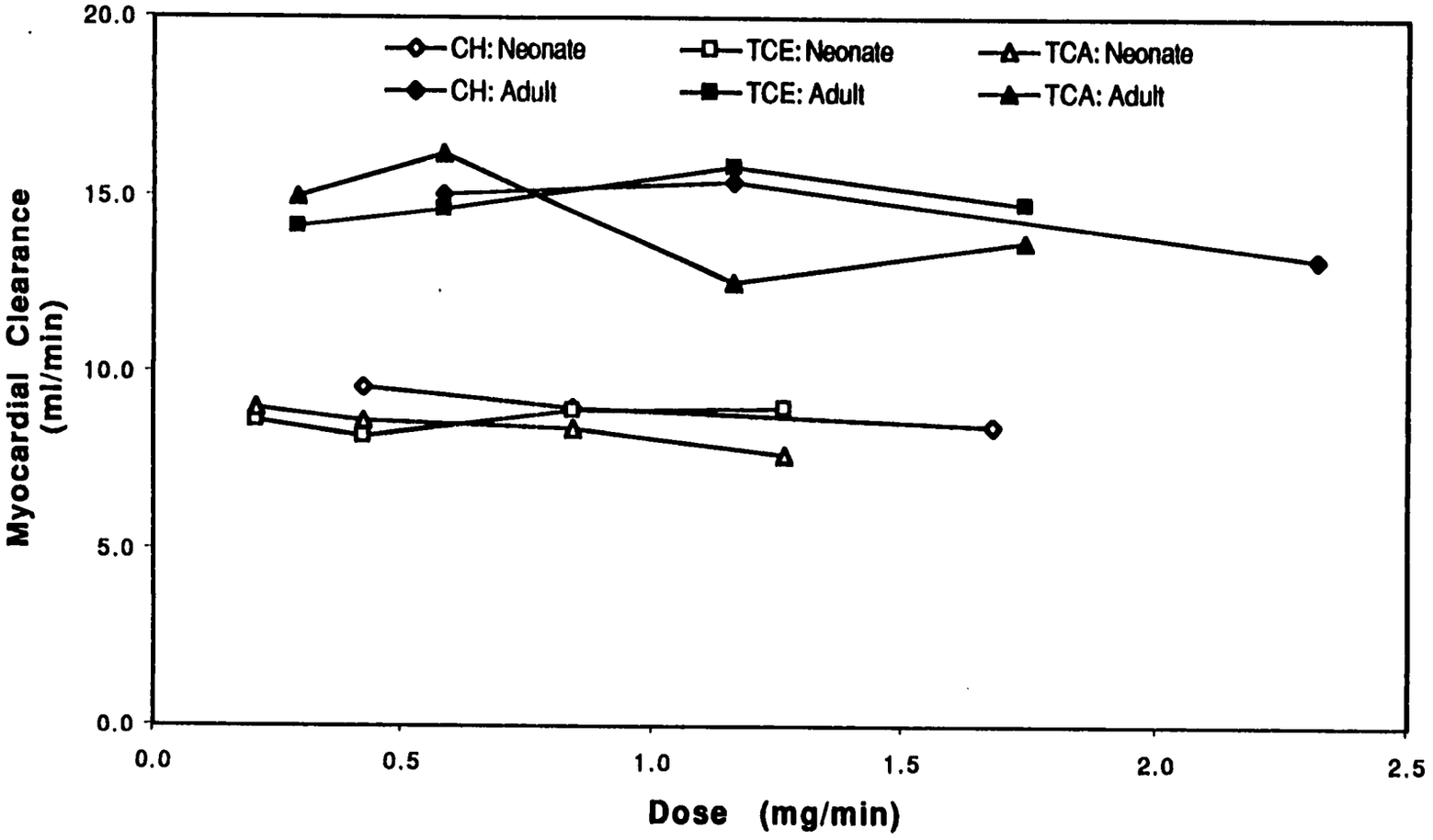


Figure 6-4. A plot of myocardial clearance *versus* dose for chloral hydrate (50, 100 and 200 µg/ml), trichloroethanol (25, 50, 100 and 150 µg/ml) and trichloroacetic acid (25, 50, 100 and 150 µg/ml) in isolated, perfused adult and neonatal rabbit hearts. Each point represents an average of 4-5 experiments.

Table 6-3. Pharmacokinetic parameters for metabolite formation after administration of various concentrations of chloral hydrate (CH), trichloroethanol (TCE) and trichloroacetic acid (TCA) in isolated perfused adult rabbit hearts. Results are expressed as the averages of 4-5 experiments.

	Chloral hydrate (μg/ml)			Trichloroethanol (μg/ml)				Trichloroacetic acid (μg/ml)			
	50	100	200	25	50	100	150	25	50	100	150
CH:											
C_{ss} (μg/ml)	-	-	-								
C_{max} (μg/ml)	-	-	-	trace[1]	trace	trace	trace	trace	trace	trace	0.115
T_{max} (min)	-	-	-								20
TCE:											
C_{ss} (μg/ml)	2.526	3.457	4.351	-	-	-	-				
C_{max} (μg/ml)	4.526	3.561	4.466	-	-	-	-	0.611	ND[2]	0.181	0.192
T_{max} (min)	20	60	50	-	-	-	-	20		10	10
TCA:											
C_{ss} (μg/ml)	0.188	0.507	0.878					-	-	-	-
C_{max} (μg/ml)	0.359	0.711	1.032	trace	trace	trace	trace	-	-	-	-
T_{max} (min)	10	60	50					-	-	-	-

[1] Indicates that the concentration was at or below the assay quantitation limits.

[2] Indicates that the analyte was not detected.

Table 6-4. Pharmacokinetic parameters for metabolite formation after administration of various concentrations of chloral hydrate (CH), trichloroethanol (TCE) and trichloroacetic acid (TCA) in isolated perfused neonatal rabbit hearts. Results are expressed as the averages of 4-5 experiments.

	Chloral hydrate (μg/ml)			Trichloroethanol (μg/ml)				Trichloroacetic acid (μg/ml)			
	50	100	200	25	50	100	150	25	50	100	150
CH:											
C_{ss} (μg/ml)	-	-	-	0.028	0.036	0.087	0.099				
C_{max} (μg/ml)	-	-	-	0.075	0.069	0.095	0.105	0.108	0.044	0.095	0.110
T_{max} (min)	-	-	-	60	20	60	50	40	40	60	120
TCE:											
C_{ss} (μg/ml)	0.133	0.243	0.219	-	-	-	-				
C_{max} (μg/ml)	0.136	0.253	0.229	-	-	-	-	ND[1]	ND	ND	ND
T_{max} (min)	40	30	40	-	-	-	-				
TCA:											
C_{ss} (μg/ml)	0.065	0.145	0.303	0.096	0.042	0.055	0.038	-	-	-	-
C_{max} (μg/ml)	0.114	0.185	0.433	0.129	0.088	0.109	0.073	-	-	-	-
T_{max} (min)	50	30	20	40	70	100	110	-	-	-	-

[1] Indicates that the analyte was not detected.

6.4 DISCUSSION AND CONCLUSIONS

Isolated organ systems have long been an integral part of the study of the direct effects of endogenous and exogenous chemicals. More recently, pharmacokinetic analysis of drug disposition in a target organ has been utilized as an additional source of information to clarify both the therapeutic effects of drugs (Askholt and Nielsen-Kudsk, 1986; Nielsen-Kudsk and Askholt, 1981; Nielsen-Kudsk and Askholt, 1987) as well as the pathogenesis of drug toxicity (He *et al.*, 1991; Tanigawara *et al.*, 1991). The present study applied the principles of non-compartmental pharmacokinetic analysis in an effort to delineate certain aspects of the disposition of CH, TCE and TCA in adult and neonatal hearts which may have important implications in their cardiac toxicity.

While equivalent concentrations of CH, TCE and TCA were administered in both adult and neonatal groups, the drug input rates for all treatments were proportionately lower in the neonatal groups because of the reduced flow rate required in the much smaller neonatal hearts. A lower rate of input would be predicted to proportionately decrease the concentration of drug at steady-state. This was not observed for any neonatal treatment group. Steady-state concentrations of CH, TCE and TCA were all significantly higher in neonatal hearts when compared to adult hearts. However, because clearance is inversely related to C_{SS}, a lower clearance would produce a proportionate increase in C_{SS}. The minimal rate of myocardial clearance of CH, TCE and TCA in neonatal hearts results in output concentrations only slightly less than their input concentrations. Lower apparent volumes of distribution for CH, TCE and TCA would also contribute to the relatively higher C_{SS} of these compounds observed in the neonatal hearts.

Mean residence time (or turn over time) is equal to the time required for approximately 63.2% of the administered dose of a drug to be eliminated from the body (Gibaldi and Perrier, 1982). The single pass MRT in an isolated organ is a function of the rate constants associated with transfer out of the

organ; therefore, the tissue retention properties of drugs can be described by their mean residence times (He *et al.*, 1991; Tanigawara *et al.*, 1991). The MRTs of CH, TCE and TCA did not differ from each other in either the adult or the neonatal hearts. The average MRTs were, however, significantly lower in the adult groups. More effective drug clearance is likely responsible for the reduced retention of these compounds in the adult hearts.

Myocardial accumulation has been estimated by the molar amounts of CH, TCE and TCA in the hearts at steady-state. The accumulation of CH, TCA and, to a lesser extent, TCE in the neonatal hearts is directly proportional to administered dose. The accumulation of CH and TCE in the adult hearts, however, exhibited disproportionate increases at higher doses. The increase in CH accumulation was exponential in nature. This property of CH and TCE is possibly related to their relatively high lipid solubilities ($K_{O/W}$ 41.04 and 26.17 for CH and TCE, respectively) which would allow unrestricted diffusion of these compounds across lipid membranes (Grüner *et al.*, 1973). While the absolute amounts (μmoles) of CH, TCE or TCA accumulated are roughly equivalent between the adult and neonatal groups, accumulation of these compounds in neonatal hearts was greater than that in adult hearts when A_{SS} was expressed on a per tissue weight basis. This observation is not easily explained; however, greater accumulation of these compounds in neonatal hearts may be related to developmental differences in the composition of cardiac tissue.

There was no difference in the rates of myocardial clearance for CH, TCE or TCA in either the adult or neonatal hearts. The clearances were, however, significantly greater in the adult *versus* neonatal hearts. At least a portion of the myocardial clearance of CH, TCE and TCA can be attributed to local metabolism of these compounds. The extent of metabolism of CH in the adult hearts appeared to be greater than that observed in neonatal hearts. However, since metabolism in individual hearts was highly variable and unpredictable no attempt was made to assess mass-balance for any of the treatments.

Non-compartmental analysis of pharmacokinetic data has gained increased acceptance in recent years (Gibaldi and Perrier, 1982; Gillespie, 1991a). The main reason for the increased use of these techniques is that summary parameter estimation can be accomplished without the necessity of nonlinear regression of the experimental data. However, the assumptions associated with the use of non-compartmental parameter estimation impose certain restrictions upon their use. It is assumed that the drug in question undergoes constant clearance, has linear first-order pharmacokinetic properties, and exhibits a terminal mono-exponential elimination phase (Gillespie, 1991a). While the clearance of CH and TCA in both adult and neonatal hearts demonstrated a slight tendency towards nonlinearity at higher doses, the slopes of the plots of clearance *versus* dose were not statistically different from zero. Therefore, the use of non-compartmental pharmacokinetic methods employing AUC and its first moment (AUMC) should provide reasonable estimates of pharmacokinetic parameters for these compounds.

The results of the present investigation indicate that the heart is a site of both accumulation and metabolism of CH, TCE and TCA in isolated, perfused adult and neonatal rabbit hearts. The ability of the heart to generate both oxidative and reductive metabolites may offer some insight into the mechanism of cardiac toxicity for CH. It is possible that the local oxidative and reductive metabolism of CH and its metabolites produces various free-radical species. Free-radicals derived from reductive dehalogenation have been implicated in the hepatic toxicity of TCA in rats and mice (Larson and Bull, 1992a; Larson and Bull, 1992b). The mutagenic potential of CH and TCA in mice has been associated with oxidative production of free-radical intermediates (Ni *et al.*, 1995; Ni *et al.*, 1994; Ni *et al.*, 1996). Lipid peroxidation resulting from free-radical attack has been described as being a fundamental mechanism of myocardial injury (Dargel, 1992; Halliwell, 1989; Loesser *et al.*, 1991; Prasad *et al.*, 1989; Thollon *et al.*, 1995). It is axiomatic that the effects of chemically reactive intermediates tend to be limited to the tissues in which they are formed. Also, very low concentrations of reactive

intermediates have demonstrated significant toxicity *in vivo*. Thus, the myocardial accumulation and local metabolism of CH, TCE and TCA may be related to the cardiac toxicity previously demonstrated in the adult rabbit heart (Fandrey *et al.*, 1992; Chapter 4).

Of particular interest, however, is the fact that cardiac toxicity was not appreciable in a similar neonatal heart model (refer to Chapter 5). The greater potential for accumulation in neonatal hearts as a result of compromised myocardial clearance would seem to suggest a potential for enhanced cardiac toxicity. Attenuation of cardiac toxicity in fetal and neonatal mammals is not, however, without precedent. The resistance of the neonatal myocardium to deleterious peroxidative effects of ischemia and subsequent reperfusion is well-established (Grice *et al.*, 1987; McCully *et al.*, 1994; Murashita *et al.*, 1992). Investigations aimed at confirming the mechanism of the apparent resistance of neonatal hearts to the cardiotoxic effects of CH and its metabolites have been undertaken (Chapter 7). Enhanced activities of key myocardial antioxidant enzymes may ameliorate the cytotoxic effects of CH and its metabolites in immature rabbit hearts.

7. DEVELOPMENTAL ASPECTS OF CHLORAL HYDRATE CARDIOTOXICITY: LIPID PEROXIDATION AND ANTI-OXIDANT STATUS

7.1 INTRODUCTION

Chloral hydrate is the oldest sedative-hypnotic still in clinical use (Butler, 1970; Hoskins, 1984). While once employed extensively in almost all patient populations, CH administration is now limited almost exclusively to pediatric and geriatric patients (Cook *et al.*, 1992; Hoskins, 1984; Marx *et al.*, 1993). However, in addition to therapeutic administration, significant environmental exposure to CH and TCA can occur inadvertently due to drinking water contamination in chlorine disinfected water supplies (U.S. Environmental Protection Agency, 1975; U.S. Environmental Protection Agency, 1982; Uden and Miller, 1983).

CH ingestion has long been associated with the development of cardiac arrhythmias, especially in situations of overdose (Graham *et al.*, 1988; Gustafson *et al.*, 1977; Hirsch and Zauder, 1986; Marshall, 1977). It has been estimated that approximately 25% of CH intoxications will result in extrasystoles (Wiseman and Hampel, 1978). However, few investigations of the direct effects of CH and/or its metabolites on the heart have been reported (Gross and Hoff, 1968; Riggs *et al.*, 1986; Trulson and Ulissey, 1987; White and Carlson, 1981).

It has been hypothesized that cardiac arrhythmias induced by CH or its metabolites may be analogous to arrhythmias produced by halogenated hydrocarbons such as halothane (Bowyer and Glasser, 1980; Brown and Cade, 1980). The mechanism of halothane-induced arrhythmias has generally been assumed to be due to sensitization of the myocardium to the effects of endogenous catecholamines (Johnston *et al.*, 1976; Steward, 1985). However, it has recently been proposed that reductive dehalogenation of

halothane, resulting in the formation of radical and carbanion complexes, may cause lipid peroxidation and the subsequent cardiotoxic effects (Gibson and Skett, 1986; Kharasch *et al.*, 1995; Spracklin *et al.*, 1995). Recent investigations have established that CH and its two phase I metabolites trichloroethanol (TCE) and trichloroacetic acid (TCA) generate free radicals with subsequent lipid peroxidation in an *in vitro* hepatic microsomal system (Ni *et al.*, 1996). It is possible that CH-related arrhythmias result from free-radical mediated lipid peroxidation in the heart.

CH, TCE and TCA all possess cardiac depressant and arrhythmogenic properties in the adult rabbit heart (Fandrey *et al.*, 1993). However, the neonatal rabbit heart is remarkably resistant to the cardiotoxic effects of these compounds (refer to Chapter 5). The objectives of the present investigation, therefore, were to determine whether cardiac arrhythmias induced by CH or its metabolites are due to formation of reactive species (ie. free radicals) in the heart, and to determine if the reduced susceptibility of immature myocardium is due to reduced induction of lipid peroxidation and/or increased activity of key myocardial antioxidant enzymes.

7.2 EXPERIMENTAL

7.2.1 Materials

7.2.1.1 Chemicals

Chloral hydrate was supplied by Bristol-Myers Squibb Canada, Inc. (Montreal, PQ). Trichloroacetic acid was obtained from Fluka Chemicals, Terochem Laboratories Ltd. (Edmonton, AB). Both CH and TCA were dried overnight under vacuum prior to use. Trichloroethanol was purchased from Aldrich Chemical Co. (Milwaukee, WI). All other reagents and solvents were purchased from either Sigma Chemical Co. (St. Louis, MO) or BDH Chemicals (Toronto, ON), and were of analytical grade.

7.2.1.2 Animals

New Zealand neonatal (7-10 day) and adult white rabbits (2-3 kg) of either sex were obtained from the licensed breeding facility at the Animal Resources Centre, University of Saskatchewan (see Appendix A, Tables A-1 and A-2). Neonatal rabbits were obtained on the morning of the experiment, and were housed in a warm (30-35°C), dark environment prior to use. All adult rabbits were housed in stainless-steel cages with access to standard rabbit laboratory chow and tap water *ad libitum*, in accordance with the recommendations of the Canadian Council on Animal Care (CCAC).

7.2.2 Methods

7.2.2.1 Cytotoxicity of CH and its metabolites

The cytotoxicity of CH, TCE and TCA *versus* K562 lymphocytes was assessed, as previously described, using the Trypan blue dye exclusion method (Prasad *et al.*, 1994). This model system was selected based on the availability and positive growth characteristics of the K562 lymphocyte cell line. Cells were incubated with CH, TCE and TCA (0, 25, 75 and 150 µg/ml) for periods of 1 and 18-20 hours. Ten microlitres of 0.2% Trypan blue in phosphate buffer was added to 10 µl of a suspension of 1 x 10^6 cells/ml and the cells were examined in a hemocytometer under light microscopy. Viable cells exclude the Trypan blue dye, while dead cells take up the dye and appear blue stained under light microscopy. Cytotoxicity of CH and its metabolites is expressed as the percent of dead cells for each treatment. Each treatment was repeated in quadruplicate.

7.2.2.2 *In vitro* incubation

Adult rabbits were sacrificed by cervical dislocation and neonatal rabbits were sacrificed by decapitation according to accepted CCAC protocols. The hearts were rapidly excised, blotted dry, and placed immediately on ice. Myocardial tissue was maintained at 4° C until incubation. Tissue from both

the left and right ventricles was minced and added to 10 volumes of 50 mM phosphate buffer (pH 7.4). The heart tissue was homogenized with a Polytron homogenizer (PT-10, Brinkman Instruments, Rexdale, ON, Canada) for two periods of 10 seconds each. The homogenate was filtered through gauze, and the resulting filtrate was used for *in vitro* incubations, protein determinations, and enzyme measurements.

Sufficient volumes of CH (150 µl of a 1 g/ml solution), TCA (150 µl of a 1 g/ml solution), or TCE (98 µl of a 1.557 g/ml solution diluted to 150 µl with phosphate buffer) were added to 1.35 ml of filtered homogenate to make a final drug concentration of 100 µg/ml (in disposable borosilicate test tubes fitted with plastic caps). Following addition of drug solution, the homogenates were vortexed and placed in a 37°C shaking water bath for a period of 60 minutes. Control incubations (150 µl phosphate buffer added) were undertaken with each group of drug incubations.

7.2.2.3 Thiobarbituric acid-reactive substances

After the 60 minute incubation, aliquots of the incubation mixtures were assayed for thiobarbituric acid-reactive substances using a modification of the method of Prasad *et al.* (Prasad *et al.*, 1992b) Briefly, 50 µL of the incubation mixture was diluted with 0.75 mL of distilled deionized water, to which was added 200 µL sodium dodecyl sulfate (8.1 % w/v) and 3.0 mL of TBA reagent (a 1:1 mixture of an aqueous 0.67% thiobarbituric acid solution and glacial acetic acid). The reaction mixture was heated at 95° C for 60 minutes. After cooling, 1.0 mL of distilled water and 5.0 ml of n-butanol:pyridine (15:1) were added, the tubes were shaken for 2 minutes using a multi-tube vortex, and were subsequently centrifuged at 3000 rpm for 15 minutes. The organic layer was used for fluorometric measurement at 553 nm with an excitation wavelength of 515 nm. Tetraethoxypropane was used as an external standard and the results were expressed as nmoles of thiobarbituric acid-reactive substances (TBARS).

7.2.2.4 Antioxidant enzyme activities

Fresh myocardial homogenate was centrifuged for 5 min at 400 x g using a refrigerated centrifuge (Beckman model J2-21). The resulting supernatant was used for the measurement of the*in vitro* activities of catalase (CAT), glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD). Enzyme activities were determined according to the method of Prasad *et al.* (1992b). Measurement of CAT activity was made using a Pye Unicam SP6-550 ultraviolet spectrophotometer. GSH-Px and SOD activities were determined using a Unicam SP1800 ultraviolet spectrophotometer with a Unicam AR25 linear recorder attached. All measurements were made at ambient temperature.

The activity of CAT was expressed as $K \cdot 10^{-3} \cdot sec^{-1} \cdot mg\ protein^{-1}$, where K is a first order rate constant for the decomposition of added hydrogen peroxide at 240 nm. GSH-Px activity was expressed as nmoles NADPH oxidized to $NADP^{+}$/minute/mg of protein (using an extinction coefficient of 6.22×10^{6}) in a reaction system containing glutathione, glutathione reductase and disodium ethylenediaminetetraacetic acid with varying volumes of supernatant. One unit of SOD activity is defined as the amount of protein that inhibits the reduction of added nitro blue tetrazolium by 50%, and was determined with the use of a standard curve.

7.2.2.5 Protocols

Rabbits were divided into 3 groups: neonatal (7-10 day), juvenile (1-2 month) and adult (5-7 month). Drug and control incubations were performed on myocardial homogenates, with 19, 11, and 12 replicates for the neonatal, juvenile and adult hearts, respectively. Enzyme activities were determined in duplicate for each heart used for drug/control incubations (N=10 neonatal, N=4 juvenile and N=4 adult). Protein determination for the control incubations and for the supernatant used in the analysis of enzyme activities was carried out according to the Biuret method (Gornall *et al.*, 1949).

TBARS results are expressed as a percentage of the respective (neonatal, juvenile or adult) control values.

7.2.2.6 Statistical Analysis

Data are presented as the mean ± S.E.M. for each group. Results were analysed by one-way or two-way (to test treatment and age effects) analysis of variance (SuperANOVA, Abacus Concepts Inc., 1989). If the analysis of variance demonstrated significant results, appropriate *post hoc* tests (Dunnett's two-tailed multiple comparison procedure and individual means comparisons) were used to identify significant treatments (Ludbrook, 1991). Correlation between variables was determined by the standard least-squares regression technique. The level of significance was accepted as $p < 0.05$.

7.3 RESULTS

7.3.1 Cytotoxicity of CH, TCE and TCA to K562 lymphocytes

The cytotoxic potential of various concentrations (0, 25, 75 and 150 µg/ml) of CH, TCE and TCA in K562 lymphocytes was evaluated by regression analysis (Figure 7-1). One hour exposure of K562 lymphocytes to increasing concentrations of CH did not significantly affect the percentage of dead cells; however, 18-20 hour exposure produced a significant increase in cytotoxicity with increasing CH concentration (p=0.008; Figure 7-1A). TCE produced a concentration-dependent increase in cytotoxicity after both 1 hour and 18-20 hour exposure in K562 lymphocytes (p=0.019 and 0.002, respectively; Figure 7-1B). One hour and 18-20 hour exposure of K562 lymphocytes to increasing concentrations of TCA also resulted in significantly enhanced cytotoxicity (p=0.027 and 0.011, respectively; Figure 7-1C).

7.3.2 Myocardial lipid peroxidation

Figure 7-2 illustrates the effects of CH, TCE and TCA (100 µg/ml) on the development of lipid peroxidation (as estimated by TBARS formation) in adult, juvenile and neonatal ventricular homogenates. Both CH (150.2% of control)

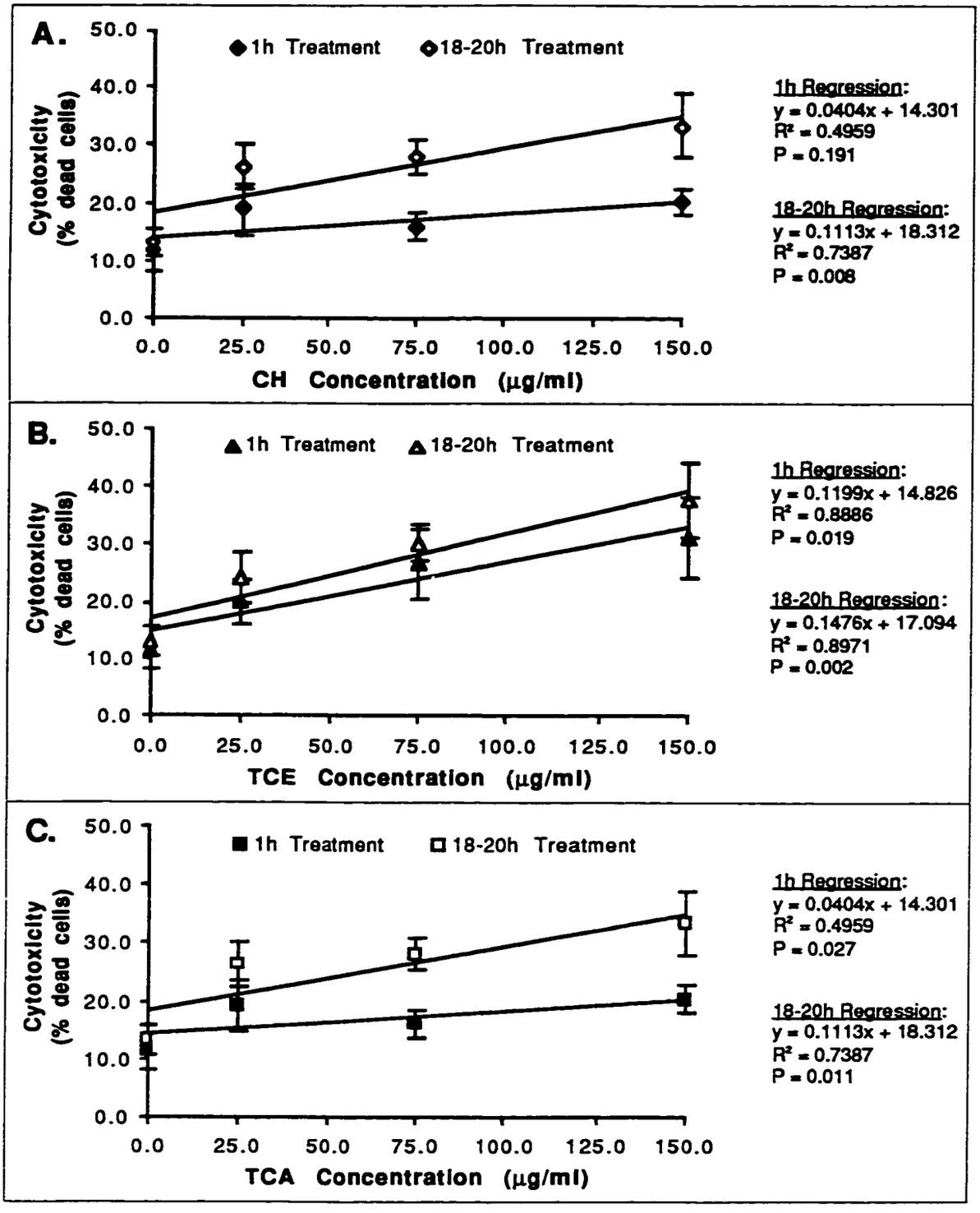


Figure 7-1. Regression analysis of the cytotoxic effects of chloral hydrate, trichloroethanol and trichloroacetic acid (0, 25, 75 and 150 µg/ml) towards K562 lymphocytes. Error bars represent the standard error of the group mean (N=4).

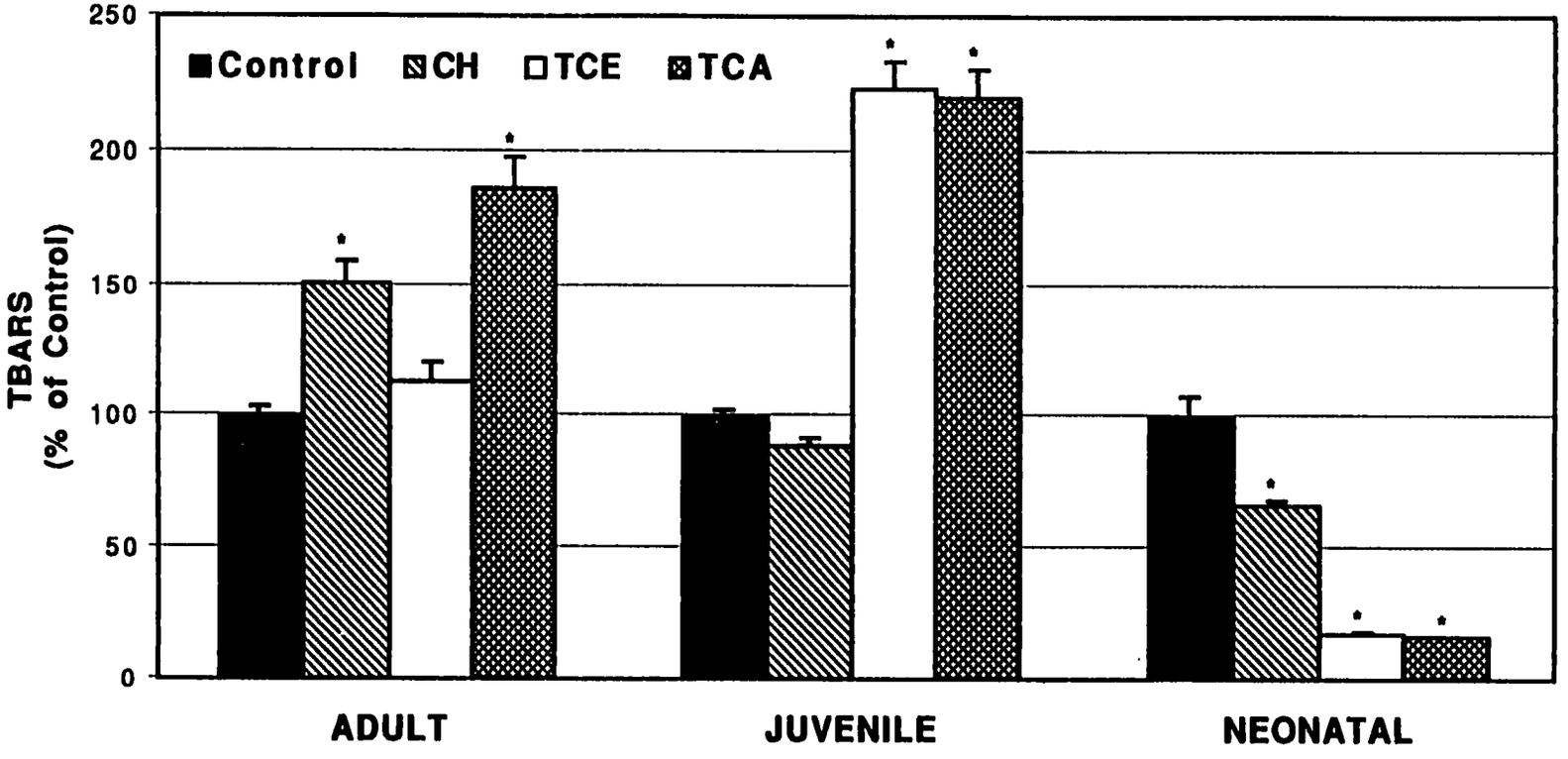


Figure 7-2. Effects of 100 µg/ml chloral hydrate (CH), trichloroethanol (TCE) and trichloroacetic acid (TCA) on the levels of thiobarbituric acid-reactive substances (TBARS) in adult, juvenile and neonatal ventricular homogenates. Results are expressed as a percentage of the respective control values (N=4 adult, N=4 juvenile and N=19 neonatal experiments; *$p<0.0001$ *vs.* respective control values).

and TCA (186.4% of control) produced significant increases in lipid peroxidation in adult hearts ($p<0.0001$). TCE and TCA were approximately equipotent with respect to lipid peroxidative effects in juvenile heart homogenates, producing increases of 223.1 and 219.6% in TBARS formation respectively ($p<0.0001$ *vs.* juvenile control). Incubation of neonatal ventricular homogenates with CH, TCE and TCA produced reductions in lipid peroxidation of 34.3, 82.4, and 83.6% respectively ($p<0.0001$ *vs.* control).

7.3.3 Antioxidant enzyme activities

The activities of catalase, glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) in neonatal, juvenile and adult ventricular tissue are described in Table 7-1. The adult and juvenile myocardial activities of catalase, GSH-Px and SOD were not significantly different from each other. However, neonatal myocardial activities of all three enzyme systems were significantly greater than the activities in the adult or juvenile groups ($p<0.0001$).

Table 7-1. Antioxidant enzyme activities in homogenates of neonatal, juvenile and adult myocardial tissue. Results are expressed as the average [S.E.M.].

	Catalase Activity (K/sec/mg protein x 10^{-3})	GSH-Px Activity (µmole NADPH/min/mg protein)	SOD Activity (U/mg protein)
Neonate (7-10 day)	25.54 [0.89]	0.165 [0.007]	100.53 [3.10]
Juvenile (1-2 month)	7.19 [0.75]	0.090 [0.002]	78.30 [2.14]
Adult (5-7 month)	7.72 [0.79]	0.112 [0.014]	74.78 [2.55]

Figure 7-3 illustrates the correlations between drug-induced lipid peroxidation and age-related enzyme activities for catalase, GSH-Px and SOD respectively (A, B, and C, respectively). Lipid peroxidation induced by TCA treatment showed significant correlations with catalase, GSH-Px and SOD activities ($p<0.05$). TCE-induced lipid peroxidation was also significantly correlated with myocardial GSH-Px activity (Figure 7-3B; $p<0.05$).

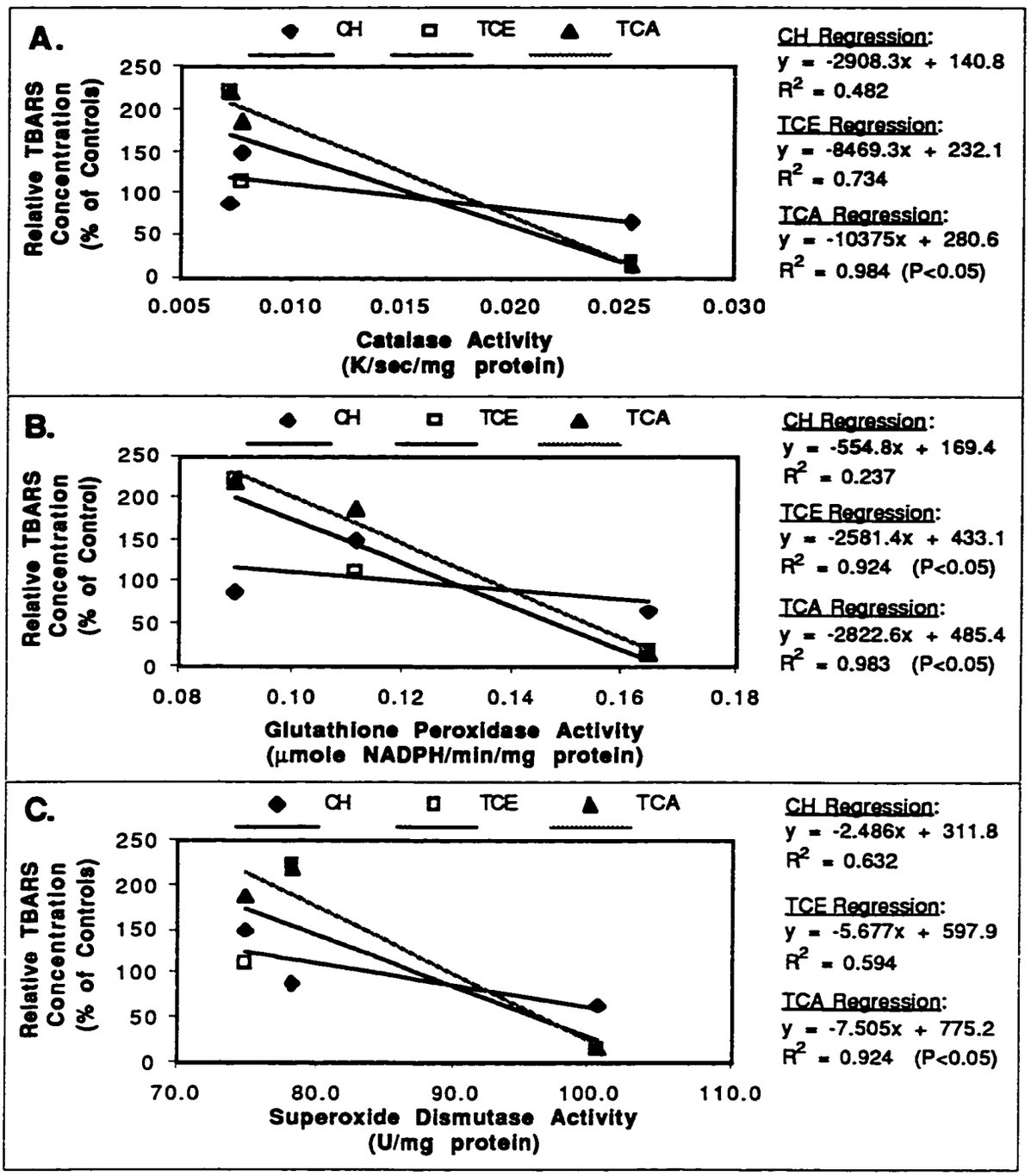


Figure 7-3. Analysis of the correlations between drug-induced lipid peroxidation (Relative TBARS concentration) and age-related enzyme activities for catalase (A), glutathione peroxidase (B) and superoxide dismutase (C). TBARS results are expressed as a percentage of the respective control values (N=4 adult, N=4 juvenile and N=19 neonatal experiments)

7.4 DISCUSSION AND CONCLUSIONS

Lipid peroxidation resulting from free-radical attack has been described as being a fundamental mechanism of myocardial injury (Dargel, 1992; Halliwell, 1989; Loesser *et al.*, 1991; Prasad *et al.*, 1989; Thollon *et al.*, 1995). For example, radical-induced lipid peroxidation has been implicated in the cardiotoxicity and arrhythmogenicity of a number of chemicals, including halothane (Gibson and Skett, 1986), adriamycin (Milei *et al.*, 1986), and isoproterenol (Singal *et al.*, 1982). Reactive oxygen species are also believed to be involved in cardiac toxicity associated with the ischemia/reperfusion phenomenon (Aiello *et al.*, 1995; Blasig *et al.*, 1994; Jeroudi *et al.*, 1994; Prasad *et al.*, 1992b).

Local cardiac metabolism and accumulation of CH, TCE and TCA has been observed in isolated, perfused adult rabbit hearts (Fandrey *et al.*, 1993). The neonatal rabbit heart is also a site of metabolism and accumulation of CH, TCE and TCA (refer to Chapter 6). It is axiomatic that the effects of chemically reactive intermediates tend to be limited to the tissues in which they are formed. Additionally, very low concentrations of reactive intermediates have demonstrated significant toxicity *in vivo.* Thus, the rabbit heart appears to be capable of producing reactive metabolites of CH, TCE and TCA which may be involved in lipid peroxidation, and subsequent cardiotoxic effects.

Plasma membrane integrity and cellular viability following chemical insult are frequently assessed by determining the ability of cultured cells to exclude trypan blue dye (Hyslop *et al.*, 1986). Initial assessment of the cytotoxic potential of CH, TCE and TCA, using the Trypan blue dye exclusion technique, demonstrated that these compounds exert concentration- and time-dependent cytotoxicity *versus* the K562 lymphocyte. These results suggest that CH, TCE and TCA adversely affect plasma membrane integrity, enabling the dye to penetrate into the cell's interior. Possible membrane-related effects include oxidative attack of polyunsaturated fatty acid side chains of the lipid bilayer.

Interaction of CH, TCE and TCA with plasma membranes has been shown to initiate lipid peroxidation in an *in vitro* hepatocyte model (Ni *et al.*, 1994; Ni *et al.*, 1996). TCA has also been shown to cause lipid peroxidation in rats and mice (Larson and Bull, 1992a). The lipid peroxidative effects of CH, TCE and TCA appear to be confirmed in the present investigation. Lipid peroxidation has been estimated by the level of thiobarbituric acid-reactive substances (TBARS) produced by the myocardial homogenates following various treatments. TBARS are a heterogeneous group of compounds formed in the decomposition of lipid peroxides, and include various aldehydes such as malondialdehyde, formaldehyde, acetaldehyde and propionaldehyde (Halliwell, 1989; Ni *et al.*, 1996). While the TBARS assay is not specific for a particular lipid peroxidation byproduct, it is sufficiently sensitive to assess the general level of lipid peroxidation produced by a chemical intervention (Ungemach, 1987).

Lipid peroxidation in the adult ventricular homogenates was most pronounced following TCA treatment. CH also produced a significant increase in the *in vitro* lipid peroxidation. TCE, however, did not appear to have any effect on lipid peroxidation in the adult tissue. Metabolic activation of TCE to TCA may be required for lipid peroxidation to occur. If levels of active metabolite (i.e., TCA) were very low following TCE administration, lipid peroxidation would not be evident. Juvenile tissue appeared to be the most susceptible to the lipoperoxidative effects of TCE and TCA. CH induced virtually no lipid peroxidation in the juvenile group. If myocardial metabolism of CH to either TCE or TCA occurred, some lipid peroxidative effects would be anticipated. This would seem to suggest that metabolic activation of CH in juvenile cardiac tissue is minimal or non-existent in this age group. It has been proposed that the lipid peroxidative effects of CH are mediated primarily through the TCA metabolite (Ni *et al.*, 1995; Ni *et al.*, 1996). TCA can be formed from oxidation of either CH or TCE (Breimer *et al.*, 1974; Marshall and Owens, 1954); therefore, TCA could mediate the lipid peroxidation occurring in either CH or TCE treatments. The strong correlation of TCA-induced lipid

peroxidation with myocardial antioxidant enzyme activities at different levels of cardiac maturity would seem to support the role of TCA as the primary mediator of oxidative injury in this model system.

Exposure of neonatal heart tissue to CH, TCE or TCA appeared to be associated with protection of the tissue from initiation of lipid peroxidation, with substantially lower TBARS formation in treated homogenates than in the control experiments. Attenuation of oxidative injury in the neonatal myocardium is not without precedent. Neonatal hearts appear to be resistant to the deleterious peroxidative effects of ischemia and subsequent reperfusion (Grice *et al.*, 1987; McCully *et al.*, 1994; Murashita *et al.*, 1992). The apparent protective effect observed in the present investigation may be related to increased activities of catalase, glutathione peroxidase and superoxide dismutase in neonatal tissue. The mechanism of tolerance of immature myocardium to ischemia is believed to be at least partially due to the enhanced radical-scavenging capacity provided by catalase (Rowland *et al.*, 1995). Higher activities of antioxidant enzyme systems have also been reported in immature lung (Frank and Groseclose, 1984; Stevens and Autor, 1977), brain (Juchau, 1990; Scarpa *et al.*, 1987), and liver (Yang *et al.*, 1993).

It may be that local (cardiac) metabolism of CH and its metabolites results in the formation of reactive species capable of causing lipid peroxidation. This phenomenon would result in a number of pathological sequelae, including intracellular calcium ion overload, which would be responsible for the demonstrated cardiotoxic effects of CH and its metabolites (Janero *et al.*, 1988; Pascoe and Reed, 1989; Trump and Berezesky, 1995). Susceptibility of the immature myocardium to these arrhythmias appears to be lower than the susceptibility of the mature myocardium. It is possible that increased myocardial antioxidant enzyme activity may play a role in the lower cardiotoxic potential of CH and its metabolites in the neonatal heart.

8. GENERAL DISCUSSION

Chloral hydrate has been described as being one of the most commonly prescribed sedative-hypnotic agents in pediatric medicine (American Academy of Pediatrics, 1993; Cook *et al.*, 1992; Green, 1993). While chloral hydrate has had a reputation as a safe therapeutic agent, anecdotal and case reports have suggested that its use in adults may be associated with the development of arrhythmias, especially in over-dose situations (Graham *et al.*, 1988; Gustafson *et al.*, 1977; Hirsch and Zauder, 1986; Marshall, 1977). Chloral hydrate-induced arrhythmias have also been documented in the pediatric population (Dean *et al.*, 1991; Hirsch and Zauder, 1986; Nordenberg *et al.*, 1971; Saarnivaara *et al.*, 1988; Seger and Schwartz, 1994). The arrhythmogenic potential of chloral hydrate, however, is unknown for either adult or pediatric patients. The present investigation was undertaken to determine the direct cardiotoxic potential of chloral hydrate in an isolated rabbit heart model, and to assess the relative susceptibility of the immature myocardium to chloral hydrate-induced cardiotoxic effects.

Any assessment of the relative vulnerability of the immature myocardium to chloral hydrate-induced arrhythmias requires knowledge of the cardiotoxic effects of this agent in mature hearts. Since the cardiotoxic potential of chloral hydrate is largely unresolved, an initial evaluation of the cardiotoxicity of the parent drug and its two primary metabolites in an isolated perfused adult rabbit heart model was carried out. This investigation demonstrated that CH, TCE and TCA all have general cardiac depressant effects in the isolated perfused adult rabbit heart at concentrations routinely encountered in clinical situations (Hindmarsh *et al.*, 1991; Mayers, 1992). The depressant effects of these agents on the coronary flow, myocardial oxygen consumption, contractility, and heart rate appeared to be largely dose-related.

All three compounds also caused significant impairment of atrio-ventricular and intra-ventricular conduction, which is associated with the development of re-entry and resultant arrhythmias (Katz, 1992). TCA administration produced the most significant and persistent conduction delays, and resulted ultimately in ventricular tachycardia and/or ventricular fibrillation at all concentrations tested. The types of ventricular tachyarrhythmias observed following TCA administration represent ECG patterns which are associated with cardiac arrest (Goldberger and Goldberger, 1990a). These findings are especially significant when one considers the persistence of TCA in humans following administration of CH (Hindmarsh *et al.*, 1991; Müller *et al.*, 1974; Sellers *et al.*, 1978).

It would be predicted from the adult heart study that situations of increased production and/or reduced clearance of TCA may be associated with significant cardiac toxicity, including the development of potentially life-threatening arrhythmias. This possibility is of particular relevance for neonatal patients, given the significant persistence of TCA in this population even after a single oral dose of chloral hydrate (Mayers *et al.*, 1991). However, while CH and its metabolites appear to be general cardiac depressants in the adult rabbit heart, these same compounds produced only minimal myocardial depression in immature hearts. Isolated neonatal rabbit hearts also proved to be remarkably resistant to alterations of myocardial rhythm and conduction. While CH, TCE and TCA produced no notable alterations in the major myocardial conduction pathways, a number of conduction defects were observed in the neonatal hearts. These atrial and ventricular ectopic beats may be related to autonomic immaturity, which is known to contribute to a relatively high incidence of ectopic beats in human newborns (Morgan *et al.*, 1965; Morgan and Guntheroth, 1965; Rogers and Richmond, 1978). It is of note that all conduction disturbances observed in neonatal hearts occurred as isolated events.

It was hypothesized that the differential toxicity of CH, TCE and TCA in adult and neonatal rabbit hearts may be due to developmental differences in

the disposition and accumulation of these compounds in the myocardium. A study of the myocardial pharmacokinetics of CH, TCE and TCA demonstrated that the heart is a site of both accumulation and metabolism of these compounds. Myocardial accumulation was estimated by the molar amounts of CH, TCE and TCA in the hearts at steady-state. The accumulation of CH, TCA and, to a lesser extent, TCE in the neonatal hearts was directly proportional to administered dose. The accumulation of CH and TCE in the adult hearts, however, exhibited disproportionate increases at higher doses. While the absolute molar amounts of CH, TCE or TCA accumulated were roughly equivalent between the adult and neonatal groups, accumulation of these compounds in neonatal hearts was greater than that in adult hearts when expressed on a per tissue weight basis. There was no difference in the rates of myocardial clearance for CH, TCE or TCA in either the adult or neonatal hearts. The clearances were, however, significantly greater in the adult than the neonatal hearts. At least a portion of the myocardial clearance of CH, TCE and TCA can be attributed to local metabolism of these compounds. The extent of metabolism of CH in the adult hearts appeared to be greater than that observed in neonatal hearts. Thus, the only pharmacokinetic basis for the enhanced cardiotoxicity of CH and its metabolites in adult rabbit hearts would appear to be the greater metabolic capacity of the mature myocardium.

The pathogenesis of chloral hydrate-associated arrhythmias has been traditionally assumed to be similar to that of halothane (Bowyer and Glasser, 1980; Brown and Cade, 1980; Byatt and Volans, 1984; DiGiovanni, 1969; Graham *et al.*, 1988; Gustafson *et al.*, 1977; Hirsch and Zauder, 1986; Katz and Epstein, 1968; Komai *et al.*, 1991; Marshall, 1977). The mechanism of halothane-induced arrhythmias was generally believed to be related to sensitization of the myocardium to the effects of endogenous catecholamines (Jastak and Pallasch, 1988; Johnston *et al.*, 1976; Katz and Epstein, 1968; Marshall, 1977; Steward, 1985). It has been demonstrated that the hypersensitivity of the newborn heart to the effects of endogenous and exogenous catecholamines commonly results in the development of

arrhythmias (Ezrin *et al.*, 1983; Friedman, 1972; Geis *et al.*, 1975; Olley and Rabinovitch, 1985; Rogers and Richmond, 1978; Rosen *et al.*, 1977). Immature hearts in the present investigation, however, proved to be highly resistant to arrhythmias and conduction disturbances following CH, TCE or TCA administration. It is unlikely, then, that sensitization of the myocardium to catecholamines plays a significant role in the development of arrhythmias induced by chloral hydrate and its metabolites. It has recently been proposed that the formation of radical and carbanion complexes with subsequent lipid peroxidation may be responsible for the cardiotoxic effects of halothane (Gibson and Skett, 1986; Kharasch *et al.*, 1995; Spracklin *et al.*, 1995). It is possible, then, that chloral hydrate-related arrhythmias result from free radical-mediated lipid peroxidation in the heart.

The ability of the heart to generate both oxidative and reductive metabolites offered some insight into the mechanism of cardiac toxicity for CH. The local oxidative and reductive metabolism of CH and its metabolites appears to produce various free-radical species. Generation of free radical species has been demonstrated for CH, TCE and TCA in various animal models (Larson and Bull, 1992a; Larson and Bull, 1992b; Ni *et al.*, 1995; Ni *et al.*, 1994; Ni *et al.*, 1996). It has been suggested that free radical-mediated lipid peroxidation represents a fundamental mechanism of myocardial injury (Dargel, 1992; Halliwell, 1989; Loesser *et al.*, 1991; Prasad *et al.*, 1989; Thollon *et al.*, 1995). The lipid peroxidative effects of CH, TCE and TCA appear to be confirmed in the present investigation. Significant increases in lipid peroxidation were observed following incubation of mature myocardial tissue with chloral hydrate or its metabolites. Lipid peroxidation in the adult ventricular homogenates was most pronounced following TCA or CH treatment, while juvenile homogenates were most susceptible to the peroxidative effects of TCA and TCE. It has been proposed that the lipid peroxidative effects of CH are mediated primarily through the TCA metabolite (Ni *et al.*, 1995; Ni *et al.*, 1996). TCA can be formed from oxidation of either CH or TCE (Breimer *et al.*, 1974; Marshall and Owens, 1954); therefore, TCA

could mediate the lipid peroxidation occurring in either CH or TCE treatments. The strong correlation of TCA-induced lipid peroxidation with myocardial antioxidant enzyme activities at different levels of cardiac maturity would seem to support the role of TCA as the primary mediator of oxidative injury in this model system.

Exposure of neonatal heart tissue to CH, TCE or TCA was associated with significant inhibition of lipid peroxidation compared with control experiments. Attenuation of oxidative injury in the neonatal myocardium is not without precedent. Neonatal hearts appear to be resistant to the deleterious peroxidative effects of ischemia and subsequent reperfusion (Grice *et al.*, 1987; McCully *et al.*, 1994; Murashita *et al.*, 1992). The apparent protective effect observed in the present investigation may be related to the higher activities of catalase, glutathione peroxidase and superoxide dismutase observed in the neonatal myocardium. The mechanism of resistance of the immature myocardium to ischemia is believed to be at least partially due to the enhanced radical-scavenging capacity provided by catalase (Rowland *et al.*, 1995). Perinatal induction of antioxidant enzyme systems has also been reported in immature lung (Frank and Groseclose, 1984; Stevens and Autor, 1977), brain (Juchau, 1990; Scarpa *et al.*, 1987), and liver (Yang *et al.*, 1993).

It is widely accepted that lipid peroxidation resulting from free-radical attack represents a fundamental mechanism of myocardial injury (Dargel, 1992; Halliwell, 1989; Loesser *et al.*, 1991; Prasad *et al.*, 1989; Thollon *et al.*, 1995). Free radical-induced lipid peroxidation has been implicated in the cardiotoxicity and arrhythmogenicity of a number of chemicals, including halothane (Gibson and Skett, 1986), adriamycin (Milei *et al.*, 1986), and isoproterenol (Singal *et al.*, 1982). Reactive oxygen species are also believed to be involved in cardiac toxicity associated with the ischemia/reperfusion phenomenon (Aiello *et al.*, 1995; Blasig *et al.*, 1994; Jeroudi *et al.*, 1994; Prasad *et al.*, 1992b). Several features of radical-induced cardiomyopathies and arrhythmias appear to be very similar to those produced by chloral hydrate and its metabolites. Myocardial stunning is believed to be a radical-

mediated manifestation of myocardial injury (Bolli, 1991; Loesser *et al.*, 1991; Prasad *et al.*, 1992a; Prasad *et al.*, 1989). CH, TCE and TCA all produced contractile dysfunction in adult rabbit hearts which appears to be analogous to the myocardial stunning observed following reperfusion of previously ischemic myocardial tissue (Bolli, 1991; Hudson, 1994; Jeroudi *et al.*, 1994; Loesser *et al.*, 1991). The conduction disturbances and arrhythmias produced by CH, TCE and TCA are consistent with electrophysiologic phenomena associated with free radical-mediated lipid peroxidation. These electrophysiological alterations include oscillations in transmembrane potential, reductions in the arrhythmogenic thresholds and abnormal automaticity, all of which predispose the myocardium to various ventricular arrhythmias (Hudson, 1994; Jeroudi *et al.*, 1994; Nakaya *et al.*, 1987; Thollon *et al.*, 1995).

Attack of subcellular targets other than lipid membranes by free radical species generated by CH, TCE and TCA would be expected to contribute to the overall cardiotoxicity of these compounds. Oxidative denaturation of protein molecules by TCA is utilized in various biochemical applications to precipitate proteins from biological preparations (Windholz, 1983). Covalent modification of protein molecules *in vivo*, however, would be expected to alter cellular homeostatic mechanisms and contribute to cellular dysfunction and eventually cell death (Bolli, 1991; Dargel, 1992; Halliwell, 1989).

Generation of non-radical products of lipid peroxidation by chloral hydrate and its metabolites could result in additional cardiotoxic effects. These toxic non-radical species consist largely of various aldehyde compounds, including acetaldehyde, malondialdehyde and 4-hydroxynonenal, which react with both the sulfhydryl groups of thiols and the amino groups of proteins (Dargel, 1992; Goedde and Agarwal, 1990; Nair *et al.*, 1986; Slater, 1984). Efficient detoxification of these carbonyl compounds is required for the maintenance of normal cell function (Goedde and Agarwal, 1990). Chloral hydrate has been shown to competitively inhibit aldehyde dehydrogenase, the enzyme primarily responsible for the oxidative biotransformation of aldehydes to acids (Berger and Weiner, 1977; Inoue *et al.*, 1979; Maki and Sladek, 1993;

Sharpe and Carter, 1993). Competitive inhibition of this important detoxification pathway would be predicted to result in the intracellular accumulation of dangerous levels of toxic carbonyl compounds which would contribute to the overall myocardial toxicity of chloral hydrate. The toxic insult initiated by chloral hydrate-mediated lipid peroxidation would thus be exacerbated by the increased production and reduced metabolic inactivation of potentially cytotoxic aldehydes.

The evidence for the involvement of chloral hydrate and/or its metabolites in free radical-mediated lipid peroxidation and subsequent cardiac toxicity in the mature heart is quite persuasive. Of particular interest, however, is the fact that radical-induced lipid peroxidation with subsequent cardiac toxicity was not appreciable in a similar neonatal heart model. The developing heart is usually characterized by excessive sensitivity to exogenous stimuli in the immediate postnatal period (Ferrer, 1977). However, a number of developmental differences in the neonatal myocardium, including immaturity of structural, biochemical and physiological components, could contribute to an overall protective effect against arrhythmias induced by CH and its metabolites. The resistance of the neonatal myocardium to deleterious peroxidative effects of ischemia and subsequent reperfusion is well-established (Grice *et al.*, 1987; McCully *et al.*, 1994; Murashita *et al.*, 1992). Neonatal hearts demonstrate better recovery of contractile function, reduced creatine kinase release and decreased left ventricular end diastolic pressure compared with adult hearts (Rowland *et al.*, 1995). The reduced susceptibility of the neonatal hearts to the cardiotoxic effects of CH, TCE or TCA appears to be analogous to the tolerance of immature myocardium to reperfusion injury. The resistance to oxidative injury in fetal and neonatal mammals is thought to be related to the activation of antioxidant defenses in the days immediately prior to parturition (Frank and Groseclose, 1984; Scarpa *et al.*, 1987; Stevens and Autor, 1977). For instance, improved tolerance to reperfusion toxicity is at least partially due to enhanced activity of catalase in the neonatal myocardium (Rowland *et al.*, 1995). It would seem that enhanced activities of key

myocardial antioxidant enzymes may be responsible for the amelioration of the cytotoxic effects of CH and its metabolites in immature rabbit hearts.

9. SUMMARY AND CONCLUSIONS

Chloral hydrate, trichloroethanol and trichloroacetic acid all exhibit cardiac depressant and arrhythmogenic properties in the isolated adult rabbit heart. Cardiac accumulation and metabolism of CH and its metabolites appears to result in the formation of reactive species capable of causing lipid peroxidation in the mature rabbit heart. This phenomenon would result in a number of pathological sequelæ, including intracellular calcium ion overload, which would be responsible for the demonstrated cardiotoxic effects of CH and its metabolites (Janero *et al.*, 1988; Pascoe and Reed, 1989; Trump and Berezesky, 1995). The relative susceptibility of the immature myocardium to CH/metabolite-induced cardiotoxicity appears to be less than that of the mature myocardium. It is possible that the demonstrated increase in the activities of several key myocardial antioxidant enzymes may play a role in the diminished neonatal cardiotoxicity of CH and its metabolites.

The resistance of healthy, term neonatal hearts to the cardiotoxic effects of chloral hydrate and its phase I metabolites may have important clinical implications. The continued use of CH as a sedative/hypnotic agent in pediatric patients would seem to pose no additional health risks for healthy patients for whom CH is administered as a single dose or in infrequent dosing regimens. It is, however, well-established that multiple-dosing of CH leads to accumulation of both TCE and TCA in immature subjects (Hindmarsh *et al.*, 1991; Reimche *et al.*, 1989). The most common indication for medium to long term use of CH is to control agitation in critically ill newborns undergoing mechanical ventilation using intermittent positive pressure (Hartley *et al.*, 1989; Mayers *et al.*, 1991; Noerr, 1992). The potential for toxic manifestations of CH in these patients cannot be considered in isolation from their general physiological immaturity and possible pre-existing medical conditions.

Of the documented CH-induced arrhythmias in the pediatric population, most involved children with compromised cardiovascular and/or pulmonary status (Hirsch and Zauder, 1986; Nordenberg *et al.*, 1971; Silver and Stier, 1971). One must consider the possibility that there exists a sub-population of pediatric patients that is susceptible to the arrhythmogenic effects of CH by virtue of pre-existing medical conditions (Hirsch and Zauder, 1986; King and England, 1987). The evidence presented establishing the cardiotoxicity of CH and its metabolites in an adult rabbit heart model would suggest that caution in the use of CH in pediatric patients with compromised cardiovascular and/or pulmonary function would be advisable.

10. FURTHER RESEARCH

While the isolated perfused heart model utilized in the present investigations has provided important insight into the direct cardiotoxic potential of CH and its metabolites, substantiation of this cardiac toxicity in an *in vivo* model should be attempted. There are numerous factors in the intact organism which can modify its response to xenobiotics. For instance, cardiovascular reflexes may mitigate all or some of the toxicity demonstrated for chloral hydrate and its metabolites in the isolated adult rabbit heart.

The rabbit is a reasonable model for the study of developmental biotransformation of xenobiotics because its long gestational period, with concomitant early differentiation of oxidative metabolism, closely resembles the situation observed in developing humans. The rabbit is also an important model for investigation of cardiac function, specifically for the investigation of the developmental aspects of drug-related cardiotoxicity *in vivo.* Demonstration of cardiotoxic effects of CH, TCE and TCA in the intact rabbit would substantiate the results obtained in the isolated perfused rabbit hearts. Of particular interest would be the relative susceptibility of the intact neonatal rabbit to the cardiotoxic effect of CH and its metabolites. These studies would provide information about the cardiotoxic potential of CH, TCE and TCA which could be extrapolated to the human clinical situation.

The specific objectives of the proposed research would be to determine: whether CH or its metabolites are involved in cardiac toxicity in intact adult and neonatal rabbits; if cardiac arrhythmias induced by CH or its metabolites in the intact rabbit are associated with the formation of reactive species (ie. free radicals) in the heart; and whether intracellular calcium ion overload is the final common pathway of the myocardial toxicity of CH and its metabolites. Clinically relevant doses/concentrations of CH, TCE and TCA should be

utilized. These have been determined either from previous human and animal studies, or from the literature.

The *in vivo* cardiotoxic potential of CH, TCE and TCA could be determined in anesthetized adult and neonatal rabbits. Aortic pressure and left ventricular pressure would be recorded to assess cardiovascular performance, and lead II ECG would be recorded to assess arrhythmias. Concentration *vs.* time profiles would be constructed for these agents over a 24 hour period. The concentrations of these agents which produce arrhythmia or cardiac depression would be determined to ascertain their potency for inducing arrhythmogenic effects. Control studies to assess the effect of the anesthetic agent on cardiac parameters would also be carried out. This study would provide information as to which of the these agents are arrhythmogenic in the intact rabbit. The relative susceptibility of the immature rabbit to the cardiotoxic effects of CH and its metabolites would also be established.

The role of myocardial lipid peroxidation and antioxidant status in the cardiac toxicity of CH, TCE, and TCA could be determined using hearts from the *in vivo* study of cardiac toxicity. Lipid peroxidation could be estimated as the level of thiobarbituric acid-reactive substances (TBARS) by the method of Prasad *et al.* (Prasad *et al.*, 1992b). If reactive oxygen species are responsible for the cardiotoxicity of CH, TCE, and TCA, then TBARS levels should be higher in hearts from treated animals compared to levels from corresponding control hearts. Additionally, the effects of pre-treatment with known free-radical scavengers on the cardiac toxicity of CH and its metabolites could be assessed. Adult rabbits would be pre-treated with appropriate doses of α-tocopherol and/or ascorbate. These animals would subsequently be treated with toxic doses of CH, TCE, or TCA, and the protective effects of the administered antioxidant(s) determined. Protection by α-tocopherol and/or ascorbate against CH/metabolite-induced cardiac toxicity would implicate free radical-mediated lipid peroxidation in their mechanism of toxicity.

The role of calcium ion (Ca^{2+}) in the cardiac toxicity of CH, TCE, and TCA could be determined in isolated perfused adult rabbit hearts. These hearts would be pre-treated with appropriate concentrations of calcium-channel blockers (amlodipine and ryanodine), phenothiazines (chlorpromazine) and calcium overload protective agents (R56865) prior to administration of toxic concentrations of CH, TCE or TCA to determine the role of intracellular Ca^{2+} in the cardiotoxic effects of these compounds. These studies would also elucidate the role of voltage-gated calcium channels on the possible intracellular accumulation of Ca^{2+}.

While *in vivo* studies may provide evidence of cardiotoxic effects of chloral hydrate and its metabolites in intact animals, the clinical significance of this information would be inconclusive. Documentation of the cardiotoxic potential of chloral hydrate in human subjects, especially pediatric patients, is required. Ethical constraints imposed on the study of potentially toxic substances in pediatric patients would preclude randomized trials of chloral hydrate in this population. However, intense cardiac monitoring of pediatric patients following multiple-dose administration of chloral hydrate would allow clinicians to assess the incidence of chloral hydrate-related arrhythmias and cardiac toxicity.

11. FUTURE DIRECTIONS

Toxicity in neonates is often a consequence of their unique pharmacokinetic processes, of which xenobiotic biotransformation is of particular importance. The ability of the developing fetus to oxidize xenobiotics relatively early in gestation may be of considerable significance with respect to toxic effects. The formation of chemically reactive intermediates (ie. free radicals, reactive oxygen species) has been implicated in the pathogenesis of numerous disease states, including: toxic necrosis; mutagenesis; teratogenesis; and chemical carcinogenesis (Radde, 1985a).

The fact that the neonatal rabbit heart demonstrates higher enzyme activities for key antioxidant enzymes is of particular interest. Whether this phenomenon is observed in other neonatal (or fetal) tissues remains to be determined. The antioxidant status of the developing human may play a key role in predicting toxicity of compounds that are capable of producing reactive oxygen species in their metabolism.

REFERENCES

Achterberg, P.W., Nieukoop, A.S., Schoutsen, B. and de Jong, J.W. Different ATP-catabolism in reperfused adult and newborn rat hearts. Am. J. Physiol. **254:** H1091-H1098, 1988.

Aiello, E.A., Jabr, R.I. and Cole, W.C. Arrhythmia and delayed recovery of cardiac action potential during reperfusion after ischemia: Role of oxygen radical-induced no-reflow phenomenon. Circ. Res. **77:** 153-162, 1995.

Alstead, S. The effect of chloral hydrate on the heart. Lancet **1:** 938-941, 1936.

American Academy of Pediatrics. Use of chloral hydrate for sedation in children. Pediatr. **92:** 471-473, 1993.

Anderson, B.J., Exarchos, H., Lee, K. and Brown, T.C.K. Oral premedication in children: a comparison of chloral hydrate, diazepam, alprazolam, midazolam, and placebo for day surgery. Anaesth. Intens. Care. **18:** 185-193, 1990.

Angus, P.W., Morgan, D.J. and Smallwood, R.A. Hypoxia and hepatic drug metabolism - clinical implications. Alim. Pharmacol. Ther. **4:** 213-225, 1990.

Anonymous. Chloral hydrate overdoses result in childrens' deaths. Drug Product Quality Review. February, 1993. The United States Pharmacopeial Convention, Inc., 1993.

Anyebuno, M.A. and Rosenfeld, C.R. Chloral hydrate toxicity in a term infant. Dev. Pharmacol. Ther. **17:** 116-120, 1991.

Artman, M., Kithas, P.A., Wike, J.S. and Strada, S.J. Inotropic responses change during postnatal maturation in rabbit. Am. J. Physiol. **255:** H335-H342, 1988.

Askholt, J. and Nielsen-Kudsk, F. Felodipine pharmacodynamics and pharmacokinetics in the isolated rabbit heart. Acta Pharmacol. Toxicol. **59:** 17-26, 1986.

Asmus, K.-D., Lal, M., Mönig, J. and Schöneich, C. Radical-induced degradation of organic halogen and sulfur compounds in oxygenated aqueous solutions. Basic Life Sci. **49:** 67-73, 1988.

Baggot, J.D. Clinical pharmacokinetics in veterinary medicine. Clin. Pharmacokin. **22:** 254-273, 1992.

Baselt, R.C., Wright, J.A. and Cravey, R.H. Therapeutic and toxic concentrations of more than 100 toxicologically significant drugs in blood, plasma, or serum: a tabulation. Clin. Chem. **21:** 44-62, 1975.

Berger, D. and Weiner, H. Effects of disulfiram and chloral hydrate on the metabolism of catecholamines in rat liver and brain. Biochem. Pharmacol. **26:** 741-747, 1977.

Berne, R.M. and Levy, M.N. Chapter 24: The Cardiac Pump. *In:* Physiology, ed. by R.M. Berne and M.N. Levy, pp. 397-416, Mosby Year Book, Toronto, 1993a.

Berne, R.M. and Levy, M.N. Chapter 31: Special Circulations. *In:* Physiology, ed. by R.M. Berne and M.N. Levy, pp. 510-531, Mosby Year Book, Toronto, 1993b.

Besunder, J.B., Reed, M.D. and Blumer, J.L. Principles of drug biodisposition in the neonate: A critical evaluation of the pharmacokinetic-pharmacodynamic interface (Part I). Clin. Pharmacokin. **14:** 189-216, 1988.

Biban, P., Baraldi, E., Pettenazzo, A., Filippone, M. and Zacchello, F. Adverse effect of chloral hydrate in two young children with obstructive sleep apnea. Pediatr. **92:** 461-463, 1993.

Blasig, I.E., Shuter, S., Garlick, P. and Slater, T. Relative time-profiles for free radical trapping, coronary flow, enzyme leakage, arrhythmias, and function during myocardial reperfusion. Free Rad. Biol. Med. **16:** 35-41, 1994.

Bolli, R. Oxygen-derived free radicals and myocardial reperfusion injury: an overview. Cardiovasc. Drugs Ther. **5:** 249-268, 1991.

Bolli, R. Myocardial ischæmia: metabolic disorder leading to cell death. Rev. Port. Cardiol. **13:** 649-653, 1994.

Bowyer, K. and Glasser, S.P. Chloral hydrate over-dose and cardiac arrhythmias. Chest. **77:** 232-235, 1980.

Bray, T.M. and Taylor, C.G. Tissue glutathione, nutrition, and oxidative stress. Can. J. Physiol. Pharmacol. **71:** 746-751, 1993.

Breimer, D.D. Clinical pharmacokinetics of hypnotics. Clin. Pharmacokin. **2:** 93-109, 1977.

Breimer, D.D., Ketelaars, H.C.J. and Van Rossum, J.M. Gas chromatographic determination of chloral hydrate, trichloroethanol, and trichloroacetic acid in blood and urine. J. Chromatogr. **88:** 55-63, 1974.

Bringmann, G., God, R., Reineis, D., Wesemann, W., Riederer, P., Rausch, W.-D., Reichmann, H. and Sontag, K.-H. The TaClo concept: 1-trichlormethyl-1,2,3,4-tetrahydro-β-carboline (TaClo), a new toxin for dopaminergic neurons. J. Neural Transm. **4:** 235-244, 1995.

Brown, A.M. and Cade, J.F. Cardiac arrhythmias after chloral hydrate overdose. Med. J. Austr. **1:** 28-29, 1980.

Brus, R. and Jacobowitz, D. The influence of norepinephrine, tyramine, and acetylcholine upon isolated perfused hearts of immature and adult rabbits. Arch. Int. Pharmacodyn. **200:** 266-272, 1972.

Butler, T.C. The introduction of chloral hydrate into medical practice. Bull. Hist. Med. **44:** 168-172, 1970.

Byatt, C. and Volans, G. Sedative and hypnotic drugs. Brit. Med. J. **289:** 1214-1217, 1984.

Carini, R., Parola, M., Dianzani, M.U. and Albano, E. Lipid peroxidation and hepatocyte death: investigation of a possible mechanism of oxidative cell injury. Ann. N.Y. Acad. Sci. **663:** 444-446, 1992.

Case, C.L., Trippel, D.L. and Gillette, P.C. New anti-arrhythmic agents in pediatrics. Pediatr. Clin. N. Am. **36:** 1293-1320, 1989.

Cerbai, E., Ambrosio, G., Porciatti, F., Chiariello, M., Giotti, A. and Mugelli, A. Cellular electrophysiological basis for oxygen radical-induced arrhythmias. Circ. **84:** 1773-1782, 1991.

Chen, F.-C.M., Yamamura, H.I. and Roeske, W.R. Ontogeny of mammalian myocardial ß-adrenergic receptors. Eur. J. Pharmacol. **58:** 255-264, 1979.

Chiu, R.C.-J. and Bindon, W. Why are newborn hearts vulnerable to global ischemia? Circ. **76:** V146-V149, 1987.

Church, S.C., Morgan, B.C., Oliver, T.K. and Guntheroth, W.G. Cardiac arrhythmias in premature infants: an indication of autonomic immaturity? J. Pediatr. **71:** 542-546, 1967.

Coltart, D.J., Spilker, B.A. and Meldrum, S.J. An electrophysiological study of human foetal cardiac muscle. Experientia. **27:** 797-799, 1971.

Cook, B.A., Bass, J.W., Nomizu, S. and Alexander, M.E. Sedation of children for technical procedures: Current standard of practice. Clin. Pediatr. **31:** 137-142, 1992.

Crebelli, R., Andreoli, C., Carere, A., Conti, L., Crochi, B., Cotta-Ramusino, M. and Benigni, R. Toxicology of halogenated aliphatic hydrocarbons: structural and molecular determinants for the disturbance of chromosome segregation and the induction of lipid peroxidation. Chem.-Biol. Interac. **98:** 113-129, 1995.

Crumb, W.J., Jr., Pigott, J.D. and Clarkson, C.W. Comparison of I_{to} in young and adult human atrial myocytes: evidence for developmental changes. Am. J. Physiol. **268:** H1335-H1342, 1995.

Curtis, M.J., Pugsley, M.K. and Walker, M.J.A. Endogenous chemical mediators of ventricular arrhythmias in ischaemic heart disease. Cardiovasc. Res. **27:** 703-719, 1993.

Cusack, B.J., Mushlin, P.S., Andrejuk, T., Voulelis, L.D. and Olson, R.D. Aging alters the force-frequency relationship and toxicity of oxidative stress in rabbit heart. Life Sci. **48:** 1769-1777, 1991.

Daniel, F.B., DeAngelo, A.B., Stober, J.A., Olson, G.R. and Page, N.P. Hepatocarcinogenicity of chloral hydrate, 2-chloroacetaldehyde, and dichloroacetic acid in the male B6C3F1 mouse. Fund. Appl. Toxicol. **19:** 159-168, 1992a.

Daniel, F.B., Robinson, M., Stober, J.A., Page, N.P. and Olson, G.R. Ninety-day toxicity study of chloral hydrate in the Sprague-Dawley rat. Drug Chem. Toxicol. **15:** 217-232, 1992b.

Dargel, R. Lipid peroxidation - a common pathogenetic mechanism? Exp. Toxic. Pathol. **44:** 169-181, 1992.

Darley-Usmar, V. and Halliwell, B. Reactive nitrogen species, reactive oxygen species, transition metal ions, and the vascular system. Pharmaceut. Res. **13:** 649-662, 1996.

Dean, R.P., Rudinsky, B.F. and Kelleher, M.D. Interaction of chloral hydrate and intravenous furosemide in a child. Clin. Pharm. **10:** 385-387, 1991.

Dekant, W., Metzler, M. and Henschler, D. Novel metabolites of trichloroethylene through dechlorination reaction in rats, mice and humans. Biochem. Pharmacol. **33:** 2021-2027, 1984.

DiGiovanni, A.J. Reversal of chloral hydrate-associated cardiac arrhythmia by a ß-adrenergic blocking agent. Anesthesiol. **31:** 93-97, 1969.

Eger, E.I., Smith, N.T., Stoetting, R.K., Cullen, D.J., Kadis, L.B. and Whitcher, C.E. Cardiovascular effects of halothane in man. Anesthesiol. **32:** 396-409, 1970.

Ezrin, A.M., Bassett, A.L. and Gelband, H. Cellular electrophysiology in the developing memmalian heart: Modification by anti-arrhythmic agents. *In:* Cardiac Arrhythmias in the Neonate, Infant, and Child, ed. by N.K. Roberts and H. Gelband, pp. 37-58, Appleton-Century-Crofts, Norwalk, Conn., 1983.

Fahrig, R., Madle, S. and Baumann, H. Genetic toxicology of trichloroethylene (TCE). Mut. Res. **340:** 1-36, 1995.

Fandrey, S.L., Gorecki, D.K.J., Semple, H.A. and Prasad, K. Disposition and accumulation of chloral hydrate and its metabolites in the isolated perfused rabbit heart. Pharm. Res. **10:** S369, 1993.

Fandrey, S.L., Hindmarsh, K.W., Gorecki, D.K.J. and Prasad, K. Effects of chloral hydrate and its metabolites on the isolated perfused rabbit heart. Pharm. Res. **9:** S277, 1992.

Ferrer, P.L. Arrhythmias in the neonate. *In:* Cardiac Arrhythmias in the Neonate, Infant, and Child, ed. by N.K. Roberts and H. Gelband, pp. 265-316, Appleton-Century-Crofts, New York, 1977.

Field, K.J., White, W.J. and Lang, C.M. Anaesthetic effects of chloral hydrate, pentobarbitone and urethane in adult male rats. Lab. Anim. **27:** 258-269, 1993.

Frank, L. and Groseclose, E.E. Preparation for birth into an O_2-rich environment: the antioxidant enzymes in the developing rabbit lung. Pediatr. Res. **18:** 240-244, 1984.

Friedman, P.J. and Cooper, J.R. The role of alcohol dehydrogenase in the metabolism of chloral hydrate. J. Pharmacol. Exp. Ther. **129:** 373-376, 1960.

Friedman, W.F. The intrinsic physiologic properties of the developing human heart. Progr. Cardiovasc. Dis. **15:** 87-111, 1972.

Friedman, W.F., Pool, P.E., Jacobowitz, D., Seagren, S.C. and Braunwald, E. Sympathetic innervation of the developing rabbit heart: Biochemical and histochemical comparison of fetal, neonatal and adult myocardium. Circ. Res. **23:** 25-32, 1968.

Ganepola, S.R. A near fatal case of corrosive burns from decomposed chloral hydrate administration. Ceyl. Med. J. **37:** 131-132, 1992.

Gardner, E. and O'Rahilly, R. The nerve supply and conducting system of the human heart at the end of the embryonic period proper. J. Anat. **121:** 571-587, 1976.

Garrett, E.R. and Lambert, H.J. Pharmacokinetics of trichloroethanol and metabolites and interconversions among variously referenced pharmacokinetic parameters. J. Pharmaceut. Sci. **62:** 550-571, 1973.

Geis, W.P., Tatooles, C.J., Priola, D.V. and Friedman, W.F. Factors influencing neurohumoral control of the heart in the newborn dog. Am. J. Physiol. **228:** 1685-1689, 1975.

Gennser, G. and Nilsson, E. Response to adrenaline, acetylcholine, and change of contraction frequency in early human hearts. Experientia. **26:** 1105-1107, 1970.

Gennser, G. and von Studnitz, W. Noradrenaline synthesis in human fetal heart. Experientia. **31:** 1422-1424, 1975.

George, J. and Farrell, G.C. Role of human hepatic cytochromes P450 in drug metabolism and toxicity. Aust. NZ J. Med. **21:** 356-362, 1991.

Gershanik, J., Boecler, B., Lertora, J.J.L., Leitner, M., Sola, A. and Kapadia, C. Monitoring levels of trichloroethanol during chloral hydrate administration to sick neonates. Clin. Res. **29:** 895A, 1981.

Gibaldi, M. and Perrier, D. Chapter 11: Noncompartmental Analysis Based on Statistical Moment Theory. *In:* Pharmacokinetics, pp. 409-418, Marcel Dekker, Inc., New York, 1982.

Gibson, G.G. and Skett, P. Introduction to Drug Metabolism. Chapman & Hall, London, 1986.

Gillespie, W.R. Noncompartmental versus compartmental modelling in clinical pharmacokinetics. Clin. Pharmacokin. **20:** 253-262, 1991a.

Gillespie, W.R. Simple methods for estimation of mean residence time and steady-state volume of distribution from continuous-infusion data. Pharmaceut. Res. **8:** 254-258, 1991b.

Gilman, J.T. and Gal, P. Pharmacokinetic and pharmacodynamic data collection in children and neonates - A quiet frontier. Clin. Pharmacokin. **23:** 1-9, 1992.

Gleich, G.J., Mongan, E.S. and Vaules, D.W. Esophageal stricture following chloral hydrate poisoning. JAMA. **201:** 120-121, 1967.

Goedde, H.W. and Agarwal, D.P. Pharmacogenetics of aldehyde dehydrogenase. Pharmacol. Ther. **45:** 345-371, 1990.

Goldberg, S.J., Lebowitz, M.D., Graver, E.J. and Hicks, S. An association of human congential cardiac malformations and drinking water contaminants. J. Am. Coll. Cardiol. **16:** 155-164, 1990.

Goldberger, A.L. and Goldberger, E. Chapter 17: Cardiac Arrest. *In:* Clinical Electrocardiography: A Simplified Approach, ed. by A.L. Goldberger and E. Goldberger, pp. 211-219, Mosby Year Book, Toronto, 1990a.

Goldberger, A.L. and Goldberger, E. Chapter 18: Bradycardias and Tachycardias - Review and Differential Diagnosis. *In:* Clinical Electrocardiography: A Simplified Approach, ed. by A.L. Goldberger and E. Goldberger, pp. 220-233, Mosby Year Book, Toronto, 1990b.

Goldsmith, J.P. Ventilatory management casebook: Chloral hydrate intoxication. J. Perinatol. **14:** 74-76, 1994.

Gorecki, D.K.J., Hindmarsh, K.W., Hall, C.A. and Mayers, D.J. Determination of chloral hydrate metabolism in adult and neonatal biological fluids after single dose administration. J. Chromatogr. **528:** 333-341, 1990.

Gornall, A.G., Bardwill, C.J. and David, M.M. Determination of serum proteins by means of biuret reaction. J. Biol. Chem. **177:** 751-766, 1949.

Graham, S.R., Day, R.O., Lee, R. and Fulde, G.W.O. Over-dose with chloral hydrate: A pharmacologic and therapeutic overview. Med. J. Austr. **149:** 686-688, 1988.

Granoff, D.M., McDaniel, D.B. and Borkowf, S.P. Cardiorespiratory arrest following aspiration of chloral hydrate. Am. J. Dis. Child. **122:** 170-171, 1971.

Green, C.J. Options for sedating children. West. J. Med. **159:** 596-597, 1993.

Green, T. and Prout, M.S. Species differences in response to trichloroethylene. II. Biotransformation in rats and mice. Tox. Appl. Pharmacol. **79:** 401-411, 1985.

Greenberg, S.B. and Faerber, E.N. Respiratory insufficiency following chloral hydrate sedtion in two children with Leigh disease (subacute necrotizing encephalomyelopathy). Pediatr. Radiol. **20:** 287-288, 1990.

Greenberg, S.B., Faerber, E.N., Aspinall, C.L. and Adams, R.C. High-dose chloral hydrate sedation for children undergoing MR imaging: Safety and efficacy in relation to age. Am. J. Roentgenol. **161:** 639-641, 1993.

Grice, W.N., Konishi, T. and Apstein, C.S. Resistance of neonatal myocardium to injury during normothermic and hypothermic ischemic arrest and reperfusion. Circ. **76:** V150-V155, 1987.

Grifka, R. and Garson, A. Arrhythmias. *In:* Neonatal Therapeutics, ed. by T.F. Yeh, pp. 164-179, Mosby Year Book, Toronto, 1991.

Gross, J.B. and Hoff, H.E. Vagal activity and cardiac arrest in chloral hydrate narcosis. Cardiovasc. Res. Center Bull. **6:** 85-98, 1968.

Grosso, M.A., Banerjee, A., St. Cyr, J.A., Rogers, K.B., Brown, J.M., Clarke, D.R., Campbell, D.N. and Harken, A.H. Cardiac 5'-nucleotidase activity increases with age and inversely relates to recovery from ischemia. J. Thor. Cardiovasc. Surg. **103:** 206-209, 1992.

Grüner, J., Krieglstein, J. and Rieger, H. Comparison of the effects of chloral hydrate and trichloroethanol on the EEG of the isolated, perfused rat brain. Naun. Schmied. Arch. Pharmacol. **277:** 333-348, 1973.

Gustafson, A., Svensson, S.-E. and Ugander, L. Cardiac arrhythmia in chloral hydrate poisoning. Acta Med. Scand. **201:** 227-230, 1977.

Halliwell, B. Free radicals, reactive oxygen species and human disease: a critical evaluation with special reference to atherosclerosis. Br. J. Exp. Path. **70:** 737-757, 1989.

Halliwell, B. Free radicals and antioxidants: A personal view. Nutr. Rev. **52:** 253-265, 1994.

Hammon, J.W., Jr. Myocardial protection in the immature heart. Ann. Thorac. Surg. **60:** 839-842, 1995.

Hara, A., Yamamoto, H., Deyashiki, Y., Nakayama, T., Oritani, H. and Sawada, H. Aldehyde dismutation catalyzed by pulmonary carbonyl reductase: kinetic studies of chloral hydrate metabolism to trichloroacetic acid and trichloroethanol. Biochim. Biophys. Acta. **1075:** 61-67, 1991.

Hartley, S., Franck, L.S. and Lundergan, F. Maintaining sedation of agitated infants in the neonatal intensive care unit with chloral hydrate: New concerns. J. Perinatol. **9:** 162-164, 1989.

Hatem, S.N., Sweeten, T., Vetter, V. and Morad, M. Evidence for presence of Ca^{2+} channel-gated Ca^{2+} stores in neonatal human atrial myocytes. Am. J. Physiol. **268:** H1195-H1201, 1995.

He, Y.-L., Tanigawara, Y., Yasuhara, M. and Hori, R. Effect of folinic acid on tissue residence and excretion of methotrexate in rats. Drug Metab. Disp. **19:** 729-734, 1991.

Hearse, D.J., Kusama, Y. and Bernier, M. Rapid electrophysiological changes leading to arrhythmias in the aerobic rat heart: photosensitization

studies with rose bengal-derived reactive oxygen intermediates. Circ. Res. **65:** 146-153, 1989.

Heller, P.F., Goldberger, B.A. and Caplan, Y.H. Chloral hydrate overdose: Trichloroethanol detection by gas chromatography/mass spectrometry. For. Sci. Int. **52:** 231-234, 1992.

Henry, P.D., Shuchleib, R., Davis, J., Weiss, E.S. and Sobel, B.E. Myocardial contracture and accumulation of mitochondrial calcium in ischemic rabbit heart. Am. J. Physiol. **233:** H677-H684, 1977.

Herren-Freund, S.L., Pereira, M.A., Khoury, M.D. and Olson, G. The carcinogenicity of trichloroethylene and its metabolites, trichloroacetic acid and dichloroacetic acid, in mouse liver. Toxicol. Appl. Pharmacol. **90:** 183-189, 1987.

Hershenson, M., Brouillette, R.T., Olsen, E. and Hunt, C.E. The effect of chloral hydrate on genioglossus and diaphragmatic activity. Pediatr. Res. **18:** 516-519, 1984.

Hindmarsh, K.W., Gorecki, D.K.J., Sankaran, K. and Mayers, D.J. Chloral hydrate administration to neonates: potential toxicological implications. Can. Soc. For. Sci. J. **24:** 239-245, 1991.

Hirsch, I.A. and Zauder, H.L. Chloral hydrate: a potential cause of arrhythmias. Anesth. Analg. **65:** 691-692, 1986.

Hobara, T., Kobayashi, H., Kawamoto, T., Iwamoto, S. and Sakai, T. Extrahepatic metabolism of chloral hydrate, trichlorethanol, and trichloroacetic acid in dogs. Pharmacol. Toxicol. **61:** 58-62, 1987.

Hoskins, B. Nonbarbiturate sedative-hypnotic agents. *In:* Toxicology of Central Nervous System Depressants, ed. by I.K. Ho, pp. 58-98, CRC Press, Boca Raton, 1984.

Hrudey, S.E., Huck, P.M., Mitton, M.J. and Kenefick, S.L. Evaluation of odour removal by pilot-scale biological treatment process trains during spring runoff in an ice-covered river. Wat. Sci. Tech. **31:** 195-201, 1995.

Hudson, K.F. A phenomenon of paradox: myocardial reperfusion injury. Heart Lung. **23:** 384-393, 1994.

Hunt, C.E., Hazinski, T.A. and Gora, P. Experimental effects of chloral hydrate on ventilatory response to hypoxia and hypercarbia. Pediatr. Res. **16:** 79-81, 1982.

Hyslop, P.A., Hinshaw, D.B., Schraufstätter, I.U., Sklar, L.A., Spragg, R.G., and Cochrane, C.G. Intracellular calcium homeostasis during hydrogen

peroxide injury to cultured P388D$_1$ cells. J. Cell. Physiol. **129:** 356-366, 1986.

Ikeda, M., Ezaki, M., Kokeguchi, S. and Ohmori, S. Studies on NADPH-dependent chloral hydrate reducing enzyme in rat liver cytosol. Biochem. Pharmacol. **30:** 1931-1939, 1981.

Ikeda, M., Hattori, H., Koyama, Y. and Ohmori, S. Determination of urochloralic acid, the glucuronic acid conjugate of TCE, by gas chromatography with electron capture detection and its application to urine, plasma, and liver. J. Chromatog. **307:** 111-119, 1984.

Ikeda, M., Miyake, Y., Ogata, M. and Ohmori, S. Metabolism of trichloroethylene. Biochem. Pharmacol. **29:** 2983-2992, 1980.

Imamura, Y., Loo, E.R. and Ichibagase, H. Effect of simultaneous administration of drugs on absorption and excretion. XVII. *In vivo* binding interaciton between sulfamethoxazole and chloral hydrate in rabbits. Chem. Pharm. Bull. **32:** 1967-1972, 1984.

Inoue, K., Nishimukai, H. and Yamasawa, K. Purification and partial characterization of aldehyde dehydrogenase from human erythrocytes. Biochim. Biophys. Acta. **569:** 117-123, 1979.

Ishii, A., Tatsunami, S., Satoh, I., Honma, T., Hamoda, H. and Yago, N. Growth dynamics of the heart from perinatal period to childhood. J. Perinatal Med. **18:** 459-463, 1990.

Janero, D.R., Burghardt, B. and Lopez, R. Protection of cardiac membrane phospholipid against oxidative injury by calcium antagonists. Biochem. Pharmacol. **37:** 4197-4203, 1988.

Jastak, J.T. and Pallasch, T. Death after chloral hydrate sedation: report of case. J. Am. Dent. Assoc. **116:** 345-348, 1988.

Jeroudi, M.O., Hartley, C.J. and Bolli, R. Myocardial reperfusion injury: role of oxygen radicals and potential therapy with antioxidants. Am. J. Cardiol. **73:** 2B-7B, 1994.

Jindrich, E.J. Toxic or tolerant: chloral and the drug dilemma. J. For. Sci. **22:** 240-241, 1977.

Johnston, R.R., Eger, E.I. and Wilson, C. A comparative interaction of epinephrine with enflurane, isoflurane, and holothane in man. Anesth. Analg. **55:** 709-712, 1976.

Juchau, M.R. Fetal and neonatal drug biotransformation. *In:* Drug Toxicity and Metabolism in Pediatrics, ed. by S. Kacew, pp. 16-34, CRC Press, Boca Raton, 1990.

Kandall, S.R., Thaler, M.M. and Erickson, R.P. Intestinal development of lysosomal and microsomal beta glucuronidase and bilirubin uridine diphosphoglucuronly transferase in normal and jaundiced rats. Pediatr. **82:** 1013-1019, 1973.

Kast, A. and Hermer, M. Beta-adrenoceptor tocolysis and effects on the heart of fetus and neonate. A review. J. Perinat. Med. **21:** 97-106, 1993.

Katz, A.M. Physiology of the Heart. Raven Press, New York, 1992.

Katz, R.L. and Epstein, R.A. The interaction of anesthetic agents and adrenergic drugs to produce cardiac arrhythmias. Anesthesiol. **29:** 763-784, 1968.

Kawamoto, T., Hobara, T., Kobayashi, H., Iwamoto, S., Sakai, T., Takano, T. and Miyazaki, Y. The metabolite ratio as a function of chloral hydrate dose and intracellular redox state in the perfused rat liver. Pharmacol. Toxicol. **60:** 325-329, 1987.

Kearns, G.L. and Reed, M.D. Clinical pharmacokinetics in infants and children - a reappraisal. Clin. Pharmacokin. **17:** 29-67, 1989.

Keeter, S., Benator, R.M., Weinberg, S.M. and Hartenberg, M.A. Sedation in pediatric CT: national survey of current practice. Ped. Radiol. **175:** 745-752, 1990.

Kharasch, E.D., Thummel, K.E., Hankins, D. and Mautz, D. Human oxidative halothane metabolism in vivo is catalyzed by P450 2E1. ISSX Proc. **8:** 94, 1995.

King, K. and England, J.F. Chloral hydrate (Noctec) overdose. Med. J. Austr. **2:** 260, 1987.

Klaunig, J.E., Ruch, R.J. and Lin, E.L.C. Effects of trichloroethylene and its metabolites on rodent hepatocyte intercellular communication. Toxicol. Appl. Pharmacol. **99:** 454-465, 1989.

Klinefelter, G.R., Suarez, J.D., Roberts, N.L. and DeAngelo, A.B. Preliminary screening for the potential of drinking water disinfection byproducts to alter male reproduction. Reprod. Toxicol. **9:** 571-578, 1995.

Komai, H., Redon, D. and Rusy, B.F. Effects of thiopental and halothane on spontaneous contractile activity induction in isolated ventricular muscle of the rabbit. Acta Anaesthesiol. Scand. **35:** 373-379, 1991.

Lambert, G.H., Muraskas, J., Andrerson, C.L. and Myers, T.F. Direct hyperbilirubinemia associated with chloral hydrate administration in the newborn. Pediatr. **86:** 277-281, 1990.

Langendorff, O. Untersuchungen am überlebenden säugethierherzen. Pflügers Arch. **61:** 291-332, 1895.

Lansky, L.L. An unusual case of childhood chloral hydrate poisoning. Am. J. Dis. Child. **127:** 275-276, 1974.

Laptook, A.R. and Rosenfeld, C.R. Chloral hydrate toxicity in a preterm infant. Pediatr. Pharmacol. **4:** 161-165, 1984.

Larson, J.L. and Bull, R.J. Metabolism and lipoperoxidative activity of trichloroacetate and dichloroacetate in rats and mice. Toxicol. Appl. Pharmacol. **115:** 268-277, 1992a.

Larson, J.L. and Bull, R.J. Species differences in the metabolism of trichoroethylene to the carcinogenic metabolites trichloroacetate and dichloroacetate. Toxicol. Appl. Pharmacol. **115:** 278-285, 1992b.

Lichenstein, R., King, J.C. and Bice, D. Evaluation of chloral hydrate for pediatric sedation. Clin. Pediatr. **32:** 632-633, 1993.

Lipshitz, M., Marino, B.L. and Sanders, S.T. Chloral hydrate side effects in young children: Causes and management. Heart & Lung. **22:** 408-414, 1993.

Loesser, K.E., Kukreja, R.C., Kazziha, S.Y., Jesse, R.L. and Hess, M.L. Oxidative damage to the myocardium: a fundamental mechanism of myocardial injury. Cardioscience. **2:** 199-216, 1991.

Lopaschuk, G.D., Collins-Nakai, R.L. and Itoi, T. Developmental changes in energy substrate use by the heart. Cardiovasc. Res. **26:** 1172-1180, 1992.

Ludbrook, J. Editorial Review: On making multiple comparisons in clinical and experimental pharmacology and physiology. Clin. Exp. Pharmacol. Physiol. **18:** 379-392, 1991.

Maki, P.A. and Sladek, N.E. Sensitivity of aldehyde dehydrogenases in murine tumor and hematopoietic progenitor cells to inhibition by chloral hydrate as determined by the ability of chloral hydrate to potentiate the cytotoxic action of mafosfamide. Biochem. Pharmacol. **45:** 231-239, 1993.

Malach, M. and Berman, N. Furosemide and chloral hydrate - adverse drug reaction. JAMA. **232:** 638-639, 1975.

Marshall, A.J. Cardiac arrhythmias caused by chloral hydrate. Brit. Med. J. **2:** 994, 1977.

Marshall, B.E. and Longnecker, D.E. General Anesthetics. *In:* Goodman and Gilman's The Pharmacological Basis of Therapeutics, ed. by A.G. Gilman, T.W. Rall, A.S. Nies and P. Taylor, pp. 285-310, Pergamon Press, New York, 1990.

Marshall, E.K. and Owens, A.H. Absorption, excretion and metabolic fate of chloral hydrate and trichloroethanol. Bull. Johns Hopkins Hosp. **95:** 1-18, 1954.

Martí-Bonmatí, L., Ronchera-Oms, C.L., Casillas, C., Poyatos, C., Torrijo, C. and Jiménez, N.V. Randomised double-blind clinical trial of intermediate- versus high-dose chloral hydrate for neuroimaging of children. Neuroradiology. **37:** 687-691, 1995.

Marx, C.M., Rosenberg, D.I., Ambuel, B., Hamlett, K.W. and Blumer, J.L. Pediatric intensive care sedation: survey of fellowship training programs. Pediatr. **91:** 369-378, 1993.

Mayers, D.J. Chloral hydrate disposition in critically ill pædiatric patients. Ph.D. Thesis. University of Saskatchewan, 1992.

Mayers, D.J., Hindmarsh, K.W., Gorecki, D.K.J. and Sankaran, K. Sedative/hypnotic effects of chloral hydrate in the neonate: trichloroethanol or parent drug? Dev. Pharmacol. Ther. **19:** 141-146, 1992.

Mayers, D.J., Hindmarsh, K.W., Sankaran, K., Gorecki, D.K.J. and Kasian, G.F. Chloral hydrate disposition following single dose administration to critically ill neonates and children. Dev. Pharmacol. Ther. **16:** 71-77, 1991.

McCully, J.D., Tsukube, T., Ataka, K., Krukenkamp, I.B., Feinberg, H. and Levitsky, S. Myocardial cytosolic calcium accumulation during ischemia/reperfusion: The effects of aging and cardioplegia. J. Card. Surg. **9:** 449-452, 1994.

Merola, C., Albarracin, C., Lebowitz, P., Bienkowski, R.S. and Barst, S.M. An audit of adverse events in children sedated with chloral hydrate or propofol during imaging studies. Pæd. Anæsth. **5:** 375-378, 1995.

Meyer, E., Van Bocxlaer, J.F., Lambert, W.E., Piette, M. and De Leenheer, A.P. Determination of chloral hydrate and metabolites in a fatal intoxication. J. Anal. Toxicol. **19:** 124-126, 1995.

Milei, J., Boveris, A., Llesuy, S., Molina, H.A., Storino, R., Ortega, D. and Milei, S.E. Amelioration of Adriamycin-induced cardiotoxicity in rabbits by prenylamine and vitamins A and E. Am. Heart J. **111:** 95-102, 1986.

Miller, R.E. and Guengerich, F.P. Metabolism of trichloroethylene in isolated hepatocytes, microsomes, and reconstituted enzyme systems containing cytochrome P450. Cancer Res. **43:** 1145-1152, 1983.

Miller, R.R. and Greenblatt, D.J. Clinical effects of chloral hydrate in hospitalized medical patients. J. Clin. Pharmacol. **19:** 669-674, 1979.

Milsap, R.L. and Szefler, S.J. Special pharmacokinetic considerations in children. *In:* Applied Pharmacokinetics of Therapeutic Drug Monitoring, ed. by W.E. Evans *et. al.,* pp. 294-330, Applied Therapeutics, Inc., Spokane, 1987.

Mindham, R.H.S. Safety of chloral. BMJ **3:** 187, 1968.

Mirkin, B.L. Ontogenesis of the adrenergic nervous system: functional and pharmacologic implications. Fed. Proc. **31:** 65-73, 1972.

Moore, P.A. Therapeutic assessment of chloral hydrate premedication for pediatric dentistry. Anesthes. Progr. **31:** 191-196, 1984.

Morgan, B.C., Bloom, R.S. and Guntheroth, W.G. Cardiac arrhythmias in premature infants. Pediatr. **135:** 658-661, 1965.

Morgan, B.C. and Guntheroth, W.G. Cardiac arrhythmias in normal newborn infants. J. Ped. **67:** 1199-1202, 1965.

Morselli, P.L. Clinical pharmacology of the perinatal period and early infancy. Clin. Pharmacokin. **17:** 13-28, 1989.

Müller, G., Spassovski, M. and Henschler, D. Metabolism of trichloroethylene in man. Ii. Pharmacokinetics of metabolites. Arch. Toxicol. **32:** 283-295, 1974.

Müller, W.F., Coulston, F. and Korte, F. Comparative metabolism of [^{14}C]trichloroethylene in chimpanzees, baboons, and rhesus monkeys. Chemosphere. **11:** 215-218, 1982.

Murashita, T., Borgers, M. and Hearse, D.J. Developmental changes in tolerance to ischaemia in the rabbit heart: Disparity between interpretations of structural, enzymatic and functional indices of injury. J. Mol. Cell. Cardiol. **24:** 1143-1154, 1992.

Nahata, M.C. Sedation in pediatric patients undergoing diagnostic procedures. Drug Intell. Clin. Pharm. **22:** 711-715, 1988.

Nair, V., Cooper, C.S., Vietti, D.E. and Turner, G.A. The chemistry of lipid peroxidation metabolites: crosslinking reactions of malondialdehyde. Lipids. **21:** 6-10, 1986.

Nakanishi, T. and Jarmakani, J.M. Developmental changes in myocardial mechanical function and subcellular organelles. Am. J. Physiol. **246:** H615-625, 1984.

Nakaya, H., Tohse, N. and Kanno, M. Electrophysiological derangements induced by lipid peroxidation in cardiac tissue. Am. J. Physiol. **253:** H1089-H1097, 1987.

National Academy of Sciences. Chapter VI. Organic Solutes. Drinking Water and Health, pp. 707-709, 1977.

Neely, J.R., Liebermeister, H., Battersby, E.J. and Morgan, H.E. Effect of pressure development on oxygen consumption by isolated rat heart. Am. J. Physiol. **212:** 804-814, 1967.

Ni, Y.-C., Kadlubar, F.F. and Fu, P.P. Formation of malondialdehyde-modified 2'-deoxyguanosinyl adduct from metabolism of chloral hydrate by mouse liver microsomes. Biochem. Biophys. Res. Comm. **216:** 1110-1117, 1995.

Ni, Y.-C., Wong, T.-Y., Kadlubar, F.F. and Fu, P.P. Hepatic metabolism of chloral hydrate to free radical(s) and induction of lipid peroxidation. Biochem. Biophys. Res. Comm. **204:** 937-943, 1994.

Ni, Y.-C., Wong, T.-Y., Lloyd, R.V., Heinze, T.M., Shelton, S., Casciano, D., Kadlubar, F.F. and Fu, P.P. Mouse liver microsomal metabolism of chloral hydrate, trichloroacetic acid, and trichloroethanol leading to induction of lipid peroxidation *via* a free radical mechanism. Drug Metab. Disp. **24:** 81-90, 1996.

Nielsen-Kudsk, F. and Askholt, J. Myocardial pharmacokinetics and pharmacodynamics of nifedipine in the isolated rabbit heart. Acta Pharmacol. Toxicol. **48:** 330-339, 1981.

Nielsen-Kudsk, F. and Askholt, J. A comparative study of the pharmacodynamics and pharmacokinetics of nicardipine and nitrendipine in the isolated rabbit heart. Pharmacol. Toxicol. **60:** 192-198, 1987.

Noerr, B. Chloral hydrate. Neon. Netw. **11:** 69-71, 1992.

Nordenberg, A., Delisle, G. and Izukawa, T. Cardiac arrhythmia in a child due to chloral hydrate ingestion. Pediatr. **47:** 134-135, 1971.

Olley, P. and Rabinovitch, M. Drugs affecting the cardiovascular system. *In:* Textbook of Pediatric Clinical Pharmacology, ed. by S.M. MacLeod and I.C. Radde, pp. 148-165, PSG Publishing Co. Ltd., Littleton, 1985.

Onks, D.L., Robertson, A.F. and Brodersen, R. The effect of chloral hydrate and its metabolites, trichloroethanol and trichloroacetic acid, on bilirubin-albumin binding. Pharmacol. Toxicol. **71:** 196-197, 1992.

Pacifici, G.M., Franchi, M., Giuliani, L. and Rane, A. Development of the glucuronyltransferase and sulphotransferase towards 2-naphthol in human fetus. Dev. Pharmacol. Ther. **14:** 108-114, 1990.

Papka, R.E. A study of catecholamine-containing cells in the hearts of fetal and postnatal rabbits by fluorescence and electron microscopy. Cell Tiss. Res. **154:** 471-484, 1974.

Pappano, A.J. Ontogenetic development of autonomic neuroeffector transmission and transmitter reactivity in embryonic and fetal hearts. Pharmacol. Rev. **29:** 3-33, 1977.

Parke, D.V., Ioannides, C. and Lewis, D.F.V. The role of the cytochromes P450 in the detoxication and activation of drugs and other chemicals. Can. J. Physiol. Pharmacol. **69:** 537-549, 1990.

Parrish, M.D., Payne, A. and Fixler, D.E. Global myocardial ischemia in the newborn, juvenile, and adult isolated isovolumic rabbit heart - Age-related differences in systolic function, diastolic stiffness, coronary resistance, myocardial oxygen consumption, and extracellular pH. Circ. Res. **61:** 609-615, 1987.

Partanen, S. and Korkala, O. Catecholamines in human fetal heart. Experientia. **30:** 798-800, 1974.

Pascoe, G.A. and Reed, D.J. Cell calcium, vitamin E, and the thiol redox system in cytoxicity. Free Rad. Biol. Med. **6:** 209-224, 1989.

Perucca, E. Drug metabolism in pregnancy, infancy and childhood. Pharmacol. Ther. **34:** 129-143, 1987.

Peters, N.S., Severs, N.J., Rothery, S.M., Lincoln, C., Yacoub, M.H. and Green, C.R. Spatiotemporal relation between gap junctions and fascia adherens junctions during postnatal development of human ventricular myocardium. Circ. Res. **90:** 713-725, 1994.

Prasad, K., Kalra, J. and Bharadwaj, B. Increased oxygen free radical activity in patients on cardiopulmonary bypass undergoing aortocoronary bypass surgery. Am. Heart J. **123:** 37-45, 1992a.

Prasad, K., Kalra, J., Chan, W.P. and Chaudhary, A.K. Effect of oxygen free radicals on cardiovascular function at organ and cellular levels. Am. Heart J. **117:** 1196-1202, 1989.

Prasad, K., Kapoor, R. and Lee, P. Purpurogallin, a scavenger of polymorphonuclear leukocyte-derived oxyradicals. Mol. Cell. Biochem. **139:** 27-32, 1994.

Prasad, K., Lee, P. and Kalra, J. Influence of endothelin on cardiovascular function, oxygen free radicals, and blood chemistry. Am. Heart J. **121:** 178-187, 1991.

Prasad, K., Lee, P., Mantha, S.V., Kalra, J., Prasad, M. and Gupta, J.B. Detection of ischemia-reperfusion cardiac injury by cardiac muscle chemiluminescence. Mol. Cell. Biochem. **115:** 49-58, 1992b.

Prout, M.S., Provan, W.M. and Green, T. Species differences in response to trichloroethylene. I. Pharmacokinetics in rats and mice. Tox. Appl. Pharmacol. **79:** 389-400, 1985.

Quantz, M., Tchervendov, C. and Chiu, R.C.-J. Unique responses of immature hearts to ischemia. J. Thorac. Cardiovasc. Surg. **103:** 927-935, 1992.

Radde, I.C. Drug metabolism. *In:* Textbook of Pediatric Clinical Pharmacology, ed. by S.M. MacLeod and I.C. Radde, pp. 56-71, PSG Publishing Co. Ltd., Littleton, 1985a.

Radde, I.C. Renal elimination of drugs during development. *In:* Textbook of Pediatric Clinical Pharmacology, ed. by S.M. MacLeod and I.C. Radde, pp. 72-87, PSG Publishing Company Ltd., Littleton, 1985b.

Rall, T.W. Hypnotics and Sedatives; Ethanol. *In:* Goodman and Gilman's The Pharmacological Basis of Therapeutics, ed. by A.G. Gilman, T.W. Rall, A.S. Nies and P. Taylor, pp. 345-382, Pergamon Press, New York, 1990.

Rane, A. Basic principles of drug disposition and action in infants and children. *In:* Pediatric Pharmacology: Therapeutic Principles in Practice, ed. by S.J. Yaffe, pp. 7-28, Academic Press, New York, 1980.

Reed, M.D. and Besunder, J.B. Developmental pharmacology: ontogenic basis of drug disposition. Ped. Clin. N. Am. **36:** 1053-1074, 1989.

Reimche, L.D., Sankaran, K., Hindmarsh, K.W., Kasian, G.F., Gorecki, D.K.J. and Tan, L. Chloral hydrate sedation in neonates and infants - clinical and pharmacologic considerations. Dev. Pharmacol. Ther. **12:** 57-64, 1989.

Riggs, T.R., Yano, Y. and Vargish, T. Opiate agonist depression of myocardial function is dose-related and independent of pentobarbital or chloral hydrate anesthesia. The Am. Surg. **52:** 654-658, 1986.

Roberts, L.A. Morphological innervation pattern of the developing rabbit heart. The Am. J. Anat. **190:** 370-384, 1991.

Rocci, M.L. and Jusko, W.J. LAGRAN program for area and moments in pharmacokinetic analysis. Comp. Prog. Biomed. **16:** 203-216, 1983.

Roffi, J. and Motelica-Heino, I. Catecholamines in fetal and neonatal rabbit heart. Experientia. **31:** 194-195, 1975.

Rogers, M.C. and Richmond, J.B. The autonomic nervous system. *In:* Perinatal Physiology, ed. by U. Stave and A.A. Weech, pp. 727-737, Plenum Medical Book Co., New York, 1978.

Rosen, M.R. and Hordof, A.J. Mechanisms of arrhythmias. *In:* Cardiac Arrhythmias in the Neonate, Infant, and Child, ed. by N.K. Roberts and H. Gelband, pp. 111-131, Appleton-Century-Crofts, New York, 1977.

Rosen, M.R., Hordof, A.J., Ilvento, J.P. and Danilo, P. Effects of adrenergic amines on electrophysiologic properties and automaticity of neonatal and adult canine Purkinje fibers: Evidence for alpha- and beta-adrenergic actions. Circ. Res. **40:** 390-400, 1977.

Rowland, R.T., Meng, X., Ao, L., Terada, L.S., Harken, A.H. and Brown, J.M. Mechanisms of immature myocardial tolerance to ischemia: Phenotypic differences in antioxidants, stress proteins, and oxidases. Surg. **118:** 446-452, 1995.

Rubanyi, G.M. Vascular effects of oxygen-derived free radicals. Free Rad. Biol. Med. **4:** 107-120, 1988.

Rumm, P.D., Takao, R.T., Fox, D.J. and Atkinson, S.W. Efficacy of sedation of children with chloral hydrate. Southern Med. J. **83:** 1040-1043, 1990.

Saarnivaara, L., Lindgren, L. and Klemola, U.-M. Comparison of chloral hydrate and midazolam orally as premedications in children undergoing otolaryngological surgery. Brit. J. Anaesth. **61:** 390-396, 1988.

Saillenfait, A.M. Developmental toxicity of trichloroethylene, tetrachloroethylene and four of their metabolites in rat whole embryo culture. Arch. Toxicol. **70:** 71-82, 1995.

Sanders, V.M., Kauffmann, B.M., White, K.L., Douglas, K.A., Barnes, D.W., Sain, L.E., Bradshaw, T.J., Borzelleca, J.F. and Munson, A.E. Toxicology

of chloral hydrate in the mouse. Envir. Health Perspec. **44:** 137-146, 1982.

Sasse, S., Brand, N.J., Kyprianou, P., Dhoot, G.K., Wade, R., Arai, M., Periasamy, M., Yacoub, M.H. and Barton, P.J.R. Troponin I gene expression during human cardiac development and in end-stage heart failure. Circ. Res. **72:** 932-938, 1993.

Scarpa, M., Rigo, A., Viglino, P., Stevanato, R., Bracco, F. and Battistin, L. Age dependence of the level of the enzymes involved in the protection against active oxygen species in the rat brain. Proc. Soc. Exp. Biol. Med. **185:** 129-133, 1987.

Schoutsen, B. and de Jong, J.W. Age-dependent increase in xanthine oxidoreductase differs in various heart cell types. Circ. Res. **61:** 604-607, 1987.

Schwieler, G.H., Douglas, J.S. and Bouhuys, A. Postnatal development of autonomic efferent innervation in the rabbit. Am. J. Physiol. **219:** 391-397, 1970.

Seger, D. and Schwartz, G. Chloral hydrate: a dangerous sedative for overdose patients? Ped. Emerg. Care **10:** 349-350, 1994.

Sellers, E.M., Carr, G., Berskin, J.G., Sellers, S. and Koch-Weser, J. Interaction of chloral hydrate and ethanol in man. II. Hemodynamics and performance. Clin. Pharmacol. Ther. **13:** 50-57, 1972a.

Sellers, E.M., Lang, M., Koch-Weser, J., LeBlanc, E. and Kalant, H. Interaction of chloral hydrate and ethanol in man. I. Metabolism. Clin. Pharmacol. Ther. **13:** 37-49, 1972b.

Sellers, E.M., Lang-Sellers, M. and Koch-Weser, J. Comparative metabolism of chloral hydrate and triclofos. J. Clin. Pharmacol. **18:** 457-461, 1978.

Sharpe, A.L. and Carter, D.E. Substrate specificity of rat liver aldehyde dehydrogenase with chloroacetaldehydes. J. Biochem. Toxicol. **8:** 155-160, 1993.

Shirkey, H. Therapeutic orphans. J. Pediatr. **72:** 119-120, 1968.

Shultz, J. and Weiner, H. Alteration of the enzymology of chloral hydrate reduction in the presence of ethanol. Biochem. Pharmacol. **28:** 3379-3384, 1979.

Silver, W. and Stier, M. Cardiac arrhythmias from chloral hydrate. Pediatr. **48:** 332-333, 1971.

Silverman, J. and Muir, W.W. A review of laboratory animal anesthesia with chloral hydrate and chloralose. Lab. An. Sci. **43:** 210-216, 1993.

Singal, P.K., Kapur, N., Dhillon, K.S., Beamish, R.E. and Dhalla, N.S. Role of free radicals in catecholamine-induced cardiomyopathy. Can. J. Physiol. Pharmacol. **60:** 1390-1397, 1982.

Slater, T.F. Free-radical mechanisms in tissue injury. Biochem. J. **222:** 1-15, 1984.

Smith, A.J. and Whyte, I.M. New drugs for old: an issue for debate? Med. J. Aust. **149:** 581-582, 1988.

Sourkes, T.L. Early clinical neurochemistry of CNS-active drugs: chloral hydrate. Mol. Chem. Neuropath. **17:** 21-30, 1992.

Spach, M.S. Changes in the topology of gap junctions as an adaptive structural response of the mycardium. Circ. Res. **90:** 1103-1106, 1994.

Spracklin, D.K., Thummel, K.E. and Kharasch, E.D. Reductive halothane metabolism in humans. ISSX Proc. **8:** 98, 1995.

Stalker, N.E., Gambertoglio, J.G., Fukumitsu, C.J., Naughton, J.L. and Benet, L.Z. Acute massive chloral hydrate intoxication treated with hemodialysis: a clinical pharmacokinetic analysis. J. Clin. Pharmacol. **18:** 136-142, 1978.

Steinberg, A.D. Should chloral hydrate be banned? Pediatr. **92:** 442-446, 1993.

Steinberg, A.D. and DeSesso, J.M. Have animal data been used inappropriately to estimate risks to humans from environmental trichloroethylene? Regul. Toxicol. Pharmacol. **18:** 137-153, 1993.

Steinberg, S.F., Rosen, T.S., Malfatto, G. and Rosen, M.R. Beta-adrenergic modulation of cardiac rhythm in a rat model of altered sympathetic neural development. J. Molec. Cell. Cardiol. **23:** 47-52, 1991.

Stevens, J.B. and Autor, A.P. Induction of superoxide dismutase by oxygen in neonatal rat lung. J. Biol. Chem. **252:** 3509-3514, 1977.

Steward, D.J. Anesthesia in childhood. *In:* Textbook of Pediatric Clinical Pharmacology, ed. by S.M. MacLeod and I.C. Radde, pp. 365-378, PSG Publishing Co. Ltd., Littleton, 1985.

Tabakoff, B., Vugrincic, C., Anderson, R. and Alivisatos, S.G.A. Reduction of chloral hydrate to trichloroethanol in brain extracts. Biochem. Pharmacol. **23:** 455-460, 1974.

Tanigawara, Y., He, Y.-L., Aiba, T., Yasuhara, M., Hori, R., Hirouchi, Y., Naganuma, H., Kawahara, Y. and Okada, R. Mean residence time in organs in toxicokinetic studies. J. Pharmacobio.-Dyn. **14:** S-40, 1991.

Thollon, C., Iliou, J.P., Cambarrat, C., Robin, F. and Vilaine, J.P. Nature of the cardiomyocyte injury induced by lipid hydroperoxides. Cardiovasc. Res. **30:** 648-655, 1995.

Toth, G.P., Kelty, K.C., George, E.L., Read, E.J. and Smith, M.K. Adverse male reproductive effects following subchronic exposure of rats to sodium dichloroacetate. Fundam. Appl. Toxicol. **19:** 57-63, 1992.

Trulson, M.E. and Ulissey, M.J. Acute administration of chloral hydrate depletes cardiac enzymes in the rat. Acta Anat. **129:** 270-274, 1987.

Trump, B.F. and Berezesky, I.K. Calcium-mediated cell injury and cell death. FASEB J. **9:** 219-228, 1995.

U.S. Environmental Protection Agency. Preliminary assessment of suspected carcinogens in drinking water. Report to Congress. U.S. E.P.A., 1975.

U.S. Environmental Protection Agency. Chemical hazard information profile. Trichloroacetaldehyde. Draft. March 31, 1982. U.S. E.P.A., Office of Toxic Substances, 1982.

Uden, P.C. and Miller, J.W. Chlorinated acids and chloral in drinking water. J. Am. Water Works Assoc. **75:** 524-526, 1983.

Ungemach, F.R. Pathobiochemical mechanisms of hepatocellular damage following lipid peroxidation. Chem. Phys. Lip. **45:** 171-205, 1987.

Urquhart, N.S. The anatomy of a study. HortSci. **16:** 621-627, 1981.

Välimäki, I. Tape recordings of the ECG in newborn infants. Acta Pæd. Scand. **S199:** 1-80, 1969.

Vannier, C., Veksler, V., Mekhfi, H., Mateo, P. and Ventura-Clapier, R. Functional tissue and developmental specificities of myofibrils and mitochondria in cardiac muscle. Can. J. Physiol. Phamacol. **74:** 23-31, 1996.

Vaz, A.D.N., Roberts, E.S. and Coon, M.J. Radical intermediates in the catalytic cycles of cytochrome P-450. Basic Life Sci. **49:** 501-507, 1988.

Vaziri, N.D., Kumar, K.P., Mirahmadi, K. and Rosen, S.M. Hemodialysis in treatment of acute chloral hydrate poisoning. South. Med. J. **70:** 377-378, 1977.

Ward, R.M. and Green, T.P. Developmental pharmacology and toxicology: Principles of study design and problems of methodology. Pharmacol. Ther. **36:** 309-334, 1988.

Weiner, M. and Newberne, J.W. Drug Metabolites in the Toxicologic Evaluation of Drug Safety. Toxicol. Appl. Pharmacol. **41:** 231-233, 1977.

White, J.F. and Carlson, G.P. Epinephrine-induced cardiac arrhythmias in rabbits exposed to trichloroethylene: Role of trichloroethylene metabolites. Tox. Appl. Pharmacol. **60:** 458-465, 1981.

Willson, R.L. Zinc and iron in free radical pathology and cellular control. *In:* Zinc in Human Biology, ed. by C.F. Mills, pp. 147-172, Springer-Verlag, New York, 1989.

Windholz, M. The Merck Index: An encyclopedia of chemicals, drugs and biologicals. 1983.

Wiseman, H.M. and Hampel, G. Cardiac arrhythmias due to chloral hydrate poisoning. Brit. Med. J. **2:** 960, 1978.

Yang, F.L., David, E.A. and DiSilvestro, R.A. Neonatal rat liver contains low concentrations of Cu-Zn superoxide dismutase protein and mRNA. Biochim. Biophys. Acta **1156:** 232-234, 1993.

Young, J.B., Vandermolen, L.A. and Pratt, C.M. Torsade de pointes: an unusual manifestation of chloral hydrate poisoning. Am. Heart J. **112:** 181-184, 1986.

Yu, B.P. Cellular defenses against damage from reactive oxygen species. Physiol. Rev. **74:** 139-162, 1994.

Zarifi, M.K., Evlogias, N., Vritsiou, M. and Theodoropoulos, V. Medium dose chloral hydrate for sedation of infants and children undergoing CT. Eur. Radiol. **5:** 524-527, 1995.

APPENDIX A

- EXPERIMENTAL SUBJECTS

Table A-1. Subject data for adult rabbit experiments (PD=pharmacodynamic; PK=pharmacokinetic).

Treatment	Weight (sem) [kg]	Heart weight (sem) [g]	PD Expts [N]	PK Expts [N]
Control	2.56 (0.12)	7.44 (0.25)	10	N/A
CH 50 µg/mL	3.09 (0.20)	9.33 (0.03)	7	4
CH 100 µg/mL	2.94 (0.21)	8.03 (0.60)	7	5
CH 200 µg/mL	2.95 (0.20)	8.47 (1.00)	6	4
TCE 25 µg/mL	2.88 (0.33)	8.48 (1.36)	6	5
TCE 50 µg/mL	3.34 (0.24)	7.21 (0.54)	7	4
TCE 100 µg/mL	2.84 (0.25)	7.04 (0.24)	7	4
TCE 150 µg/mL	2.76 (0.22)	6.83 (0.45)	6	5
TCA 25 µg/mL	2.64 (0.10)	6.75 (0.27)	6	5
TCA 50 µg/mL	2.64 (0.22)	6.18 (0.40)	7	4
TCA 100 µg/mL	2.83 (0.39)	7.24 (0.58)	6	4
TCA 150 µg/mL	2.78 (0.16)	6.53 (0.45)	6	5

Table A-2. Subject data for neonatal rabbit experiments (PD=pharmacodynamic; PK=pharmacokinetic).

Treatment	Weight (sem) [kg]	Heart weight (sem) [g]	PD Expts [N]	PK Expts [N]
Control	146.1 (10.8)	0.96 (0.07)	12	N/A
CH 50 µg/mL	124.5 (11.1)	0.98 (0.06)	6	4
CH 100 µg/mL	135.9 (20.0)	0.91 (0.08)	6	4
CH 200 µg/mL	175.7 (13.9)	0.96 (0.05)	7	5
TCE 25 µg/mL	132.7 (11.0)	0.89 (0.08)	6	4
TCE 50 µg/mL	141.0 (9.4)	0.78 (0.07)	6	4
TCE 100 µg/mL	151.9 (9.4)	0.92 (0.05)	6	4
TCE 150 µg/mL	134.9 (17.4)	0.83 (0.09)	7	4
TCA 25 µg/mL	189.8 (11.4)	1.00 (0.06)	6	4
TCA 50 µg/mL	167.0 (5.2)	0.89 (0.03)	6	4
TCA 100 µg/mL	135.8 (4.9)	0.74 (0.03)	7	4
TCA 150 µg/mL	213.1 (26.3)	1.17 (0.13)	7	4

APPENDIX B

- REPRESENTATIVE ADULT PHYSIOLOGIC TRACINGS
- SUMMARIES OF ADULT PHYSIOLOGIC PARAMETERS
- MAXIMAL ADULT PHARMACODYNAMIC EFFECTS

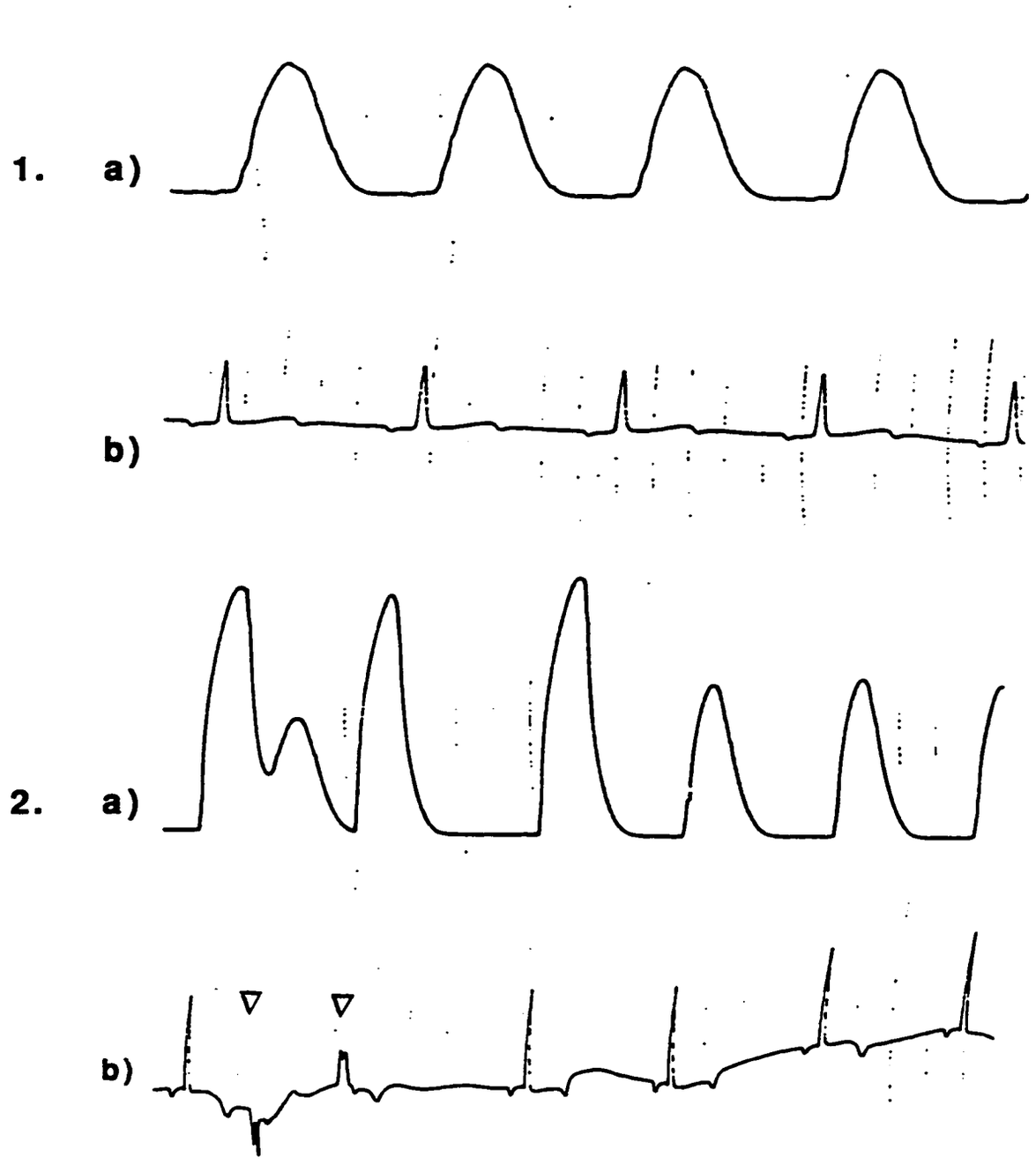


Figure B-1. Simultaneous left ventricular pressure (a) and lead I ECG (b) tracings of a representative chloral hydrate treated (200 µg/ml) adult rabbit heart (sensitivity=100 mV/mm; chart speed=50 mm/s in #1 and 25 mm/s in #2). 1(a) and (b) Pressure and ECG indices are normal at T=0 min. of the protocol. 2(b) Note the development of multifocal premature ventricular contractions at T=100-110 min. of the protocol.

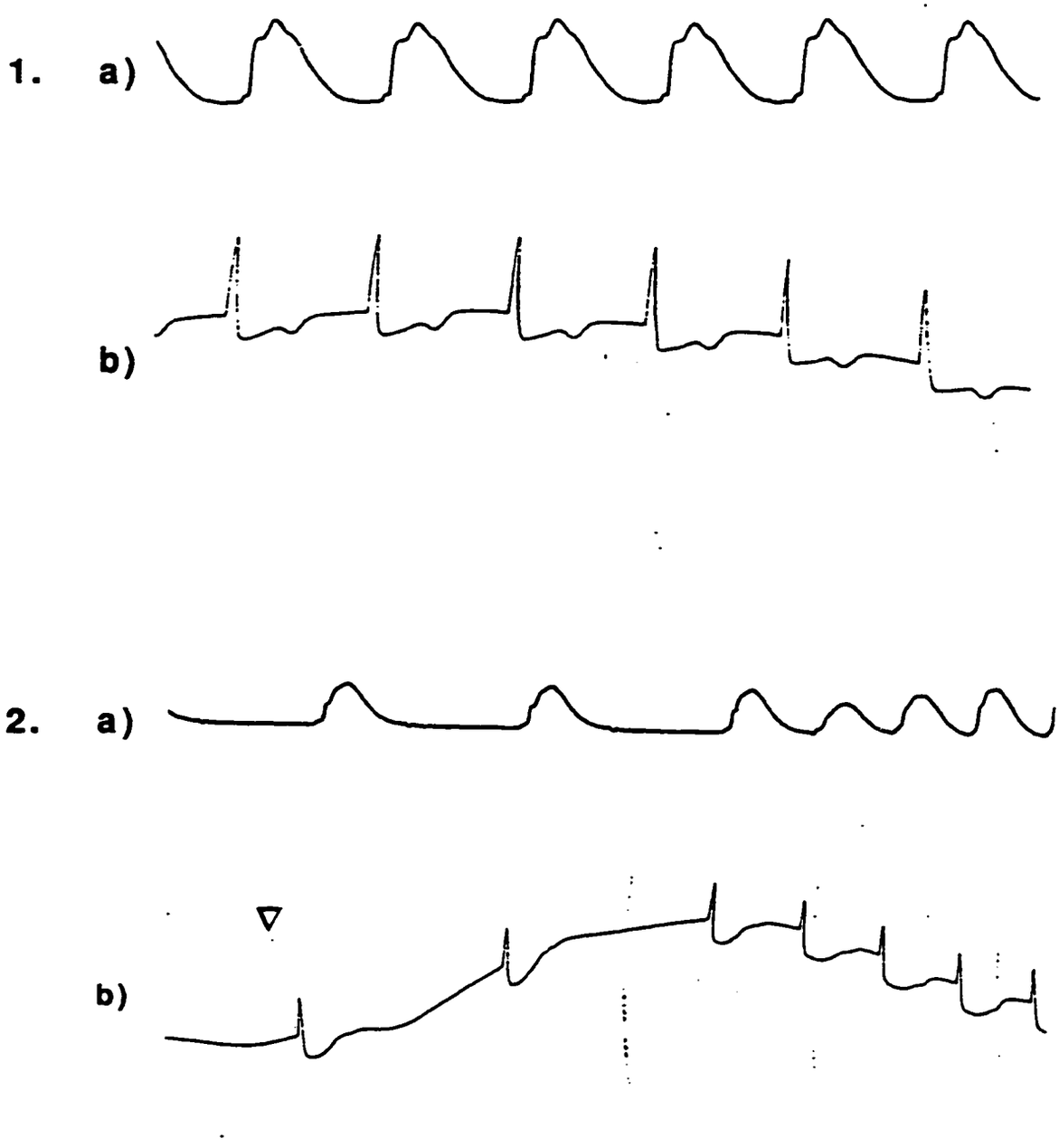


Figure B-2. Simultaneous left ventricular pressure (a) and lead I ECG (b) tracings of a representative trichloroethanol treated (100 μg/ml) adult rabbit heart (sensitivity=100 mV/mm; chart speed=50 mm/s in #1 and 25 mm/s in #2). 1(a) and (b) illustrate normal pressure and ECG indices at T=0 of the protocol. 2(b) Note the development of sinus bradycardia at T=15 min. of the protocol.

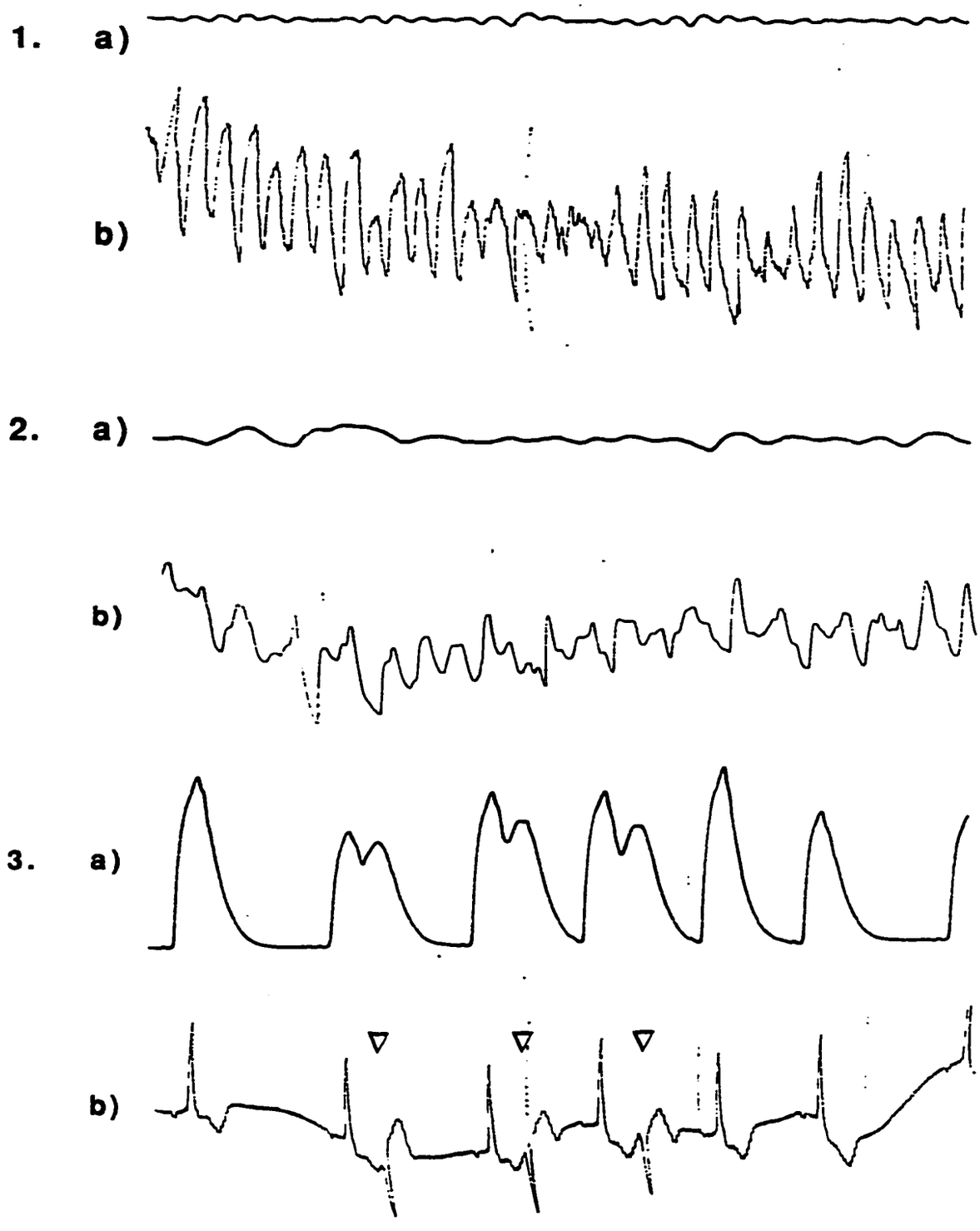


Figure B-3. Simultaneous left ventricular pressure (a) and lead I ECG (b) tracings of a representative trichloroacetic acid treated (50 µg/ml) adult rabbit heart in which serious ventricular arrhythmias developed (sensitivity=100 mV/mm; chart speed= 25 mm/s). 1(b) Ventricular tachycardia @ T=60 min. 2(b) Torsade de pointes @ T=65 min. 3(b) Ventricular bigeminy @ T=75 min.

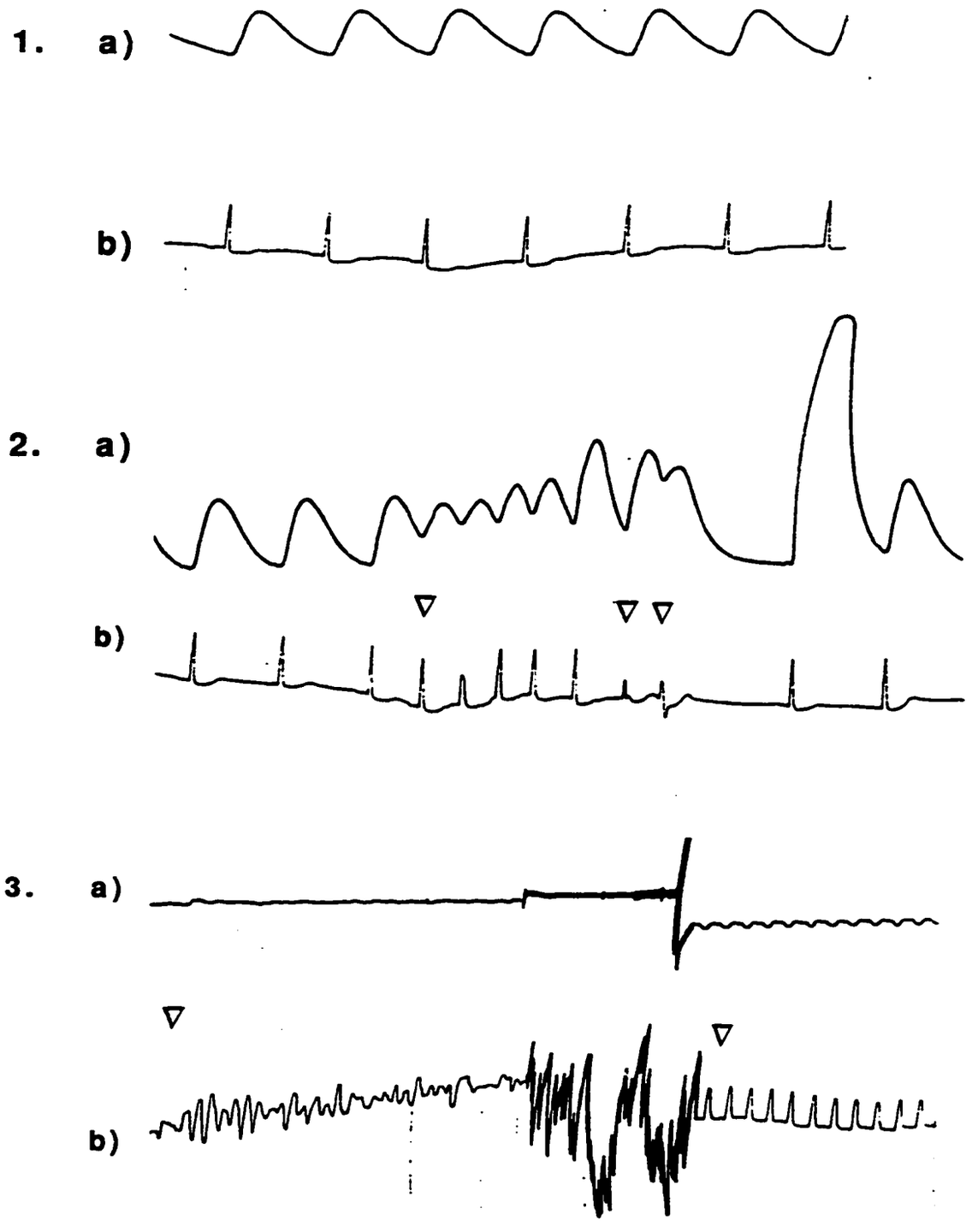


Figure B-4. Simultaneous left ventricular pressure (a) and lead I ECG (b) tracings of a representative trichloroacetic acid treated (50 μg/ml) adult rabbit heart (sensitivity=100 mV/mm; chart speed= 25 mm/s). 1(a) and (b) illustrate normal pressure and ECG indices at T=0 of the protocol. 2(b) Triggered activity with PVC's @ T=20 min. 3(b) Ventricular fibrillation which converted to ventricular tachycardia @ T=70-75 min.

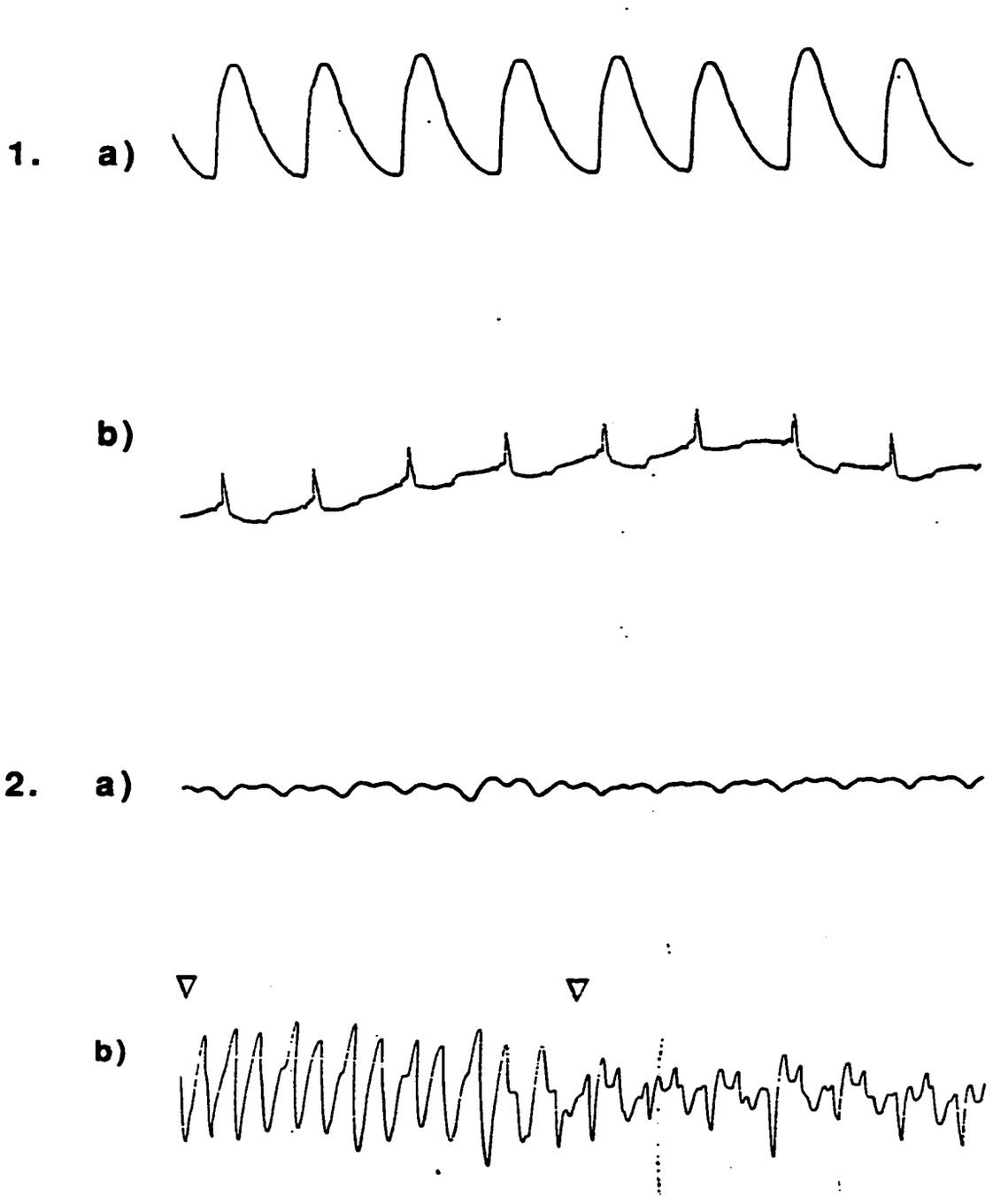


Figure B-5. Simultaneous left ventricular pressure (a) and lead I ECG (b) tracings of a representative trichloroacetic acid treated (150 µg/ml) adult rabbit heart (sensitivity=100 mV/mm; chart speed=25 mm/s) in which serious ventricular arrhythmias developed. 1(a) and (b) illustrate normal pressure and ECG indices at T=0 of the protocol. 2(b) Note the development of ventricular tachycardia at T=35 min. of the protocol.

Table B-1. Summary of the physiological parameters for adult control experiments, expressed as a fraction of baseline values (N=10).

Time	CF		HR		DP		MVO_2		dP/dT		PR Interval		QRS Duration	
	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE
0	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000
10	0.951	0.024	1.045	0.026	1.005	0.038	1.046	0.053	1.087	0.096	0.975	0.025	1.016	0.045
20	0.872	0.037	1.040	0.028	0.937	0.032	0.966	0.028	0.965	0.092	1.025	0.025	0.920	0.057
30	0.915	0.024	0.992	0.011	1.018	0.058	0.983	0.039	1.007	0.085	1.021	0.021	1.031	0.049
40	0.915	0.025	0.990	0.033	1.031	0.058	1.010	0.057	1.049	0.071	1.000	0.000	0.941	0.052
50	0.912	0.032	0.993	0.023	1.055	0.063	1.039	0.057	1.107	0.092	1.033	0.055	0.966	0.062
60	0.904	0.021	0.940	0.023	1.083	0.063	1.010	0.060	1.081	0.089	1.038	0.066	0.977	0.044
70	0.890	0.030	0.998	0.023	1.019	0.037	1.009	0.027	1.108	0.142	1.064	0.027	1.010	0.057
80	0.887	0.029	0.988	0.013	1.096	0.057	1.073	0.044	1.080	0.100	1.144	0.096	1.009	0.060
90	0.830	0.040	0.982	0.032	1.062	0.066	1.025	0.033	1.041	0.150	1.066	0.059	0.956	0.057
100	0.841	0.031	0.997	0.019	1.058	0.052	1.049	0.054	1.082	0.174	1.087	0.046	1.016	0.062
110	0.851	0.041	0.993	0.032	1.044	0.071	1.015	0.057	1.073	0.211	1.045	0.045	0.892	0.050
120	0.847	0.034	1.010	0.028	1.068	0.064	1.065	0.053	1.042	0.126	1.073	0.043	0.980	0.023

Table B-2. Summary of the physiological parameters for adult chloral hydrate 50 µg/ml experiments, expressed as a fraction of baseline values (N=7).

Time	CF		HR		DP		MVO_2		dP/dT		PR Interval		QRS Duration	
(min.)	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE
0	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000
10	0.952	0.034	0.941	0.049	0.959	0.031	0.900	0.044	0.858	0.042	1.099	0.075	0.999	0.042
20	0.920	0.028	0.908	0.028	0.868	0.063	0.796	0.076	0.720	0.066	1.228	0.119	1.055	0.110
30	0.900	0.047	0.948	0.050	0.819	0.050	0.782	0.072	0.737	0.100	1.290	0.121	1.131	0.049
40	0.819	0.059	0.907	0.056	0.797	0.055	0.737	0.089	0.618	0.070	1.190	0.102	1.149	0.064
50	0.816	0.054	0.853	0.040	0.804	0.054	0.693	0.065	0.655	0.095	1.200	0.104	1.093	0.051
60	0.785	0.058	0.913	0.053	0.826	0.067	0.766	0.092	0.669	0.099	1.119	0.079	1.085	0.063
70	0.938	0.080	0.992	0.053	0.857	0.053	0.854	0.074	0.833	0.087	1.203	0.134	1.041	0.033
80	0.955	0.056	0.997	0.071	0.926	0.071	0.913	0.069	0.893	0.074	1.179	0.105	1.046	0.040
90	0.905	0.051	0.954	0.046	0.978	0.067	0.933	0.077	0.774	0.078	1.015	0.025	1.079	0.063
100	0.866	0.034	0.955	0.045	0.914	0.060	0.876	0.077	0.780	0.099	0.974	0.060	0.947	0.081
110	0.854	0.040	0.910	0.047	0.958	0.073	0.862	0.076	0.772	0.102	1.111	0.097	0.964	0.100
120	0.856	0.053	0.900	0.047	0.944	0.082	0.845	0.083	0.740	0.081	1.061	0.096	1.017	0.063

Table B-3. Summary of the physiological parameters for adult chloral hydrate 100 µg/ml experiments, expressed as a fraction of baseline values (N=7).

Time	CF		HR		DP		MVO_2		dP/dT		PR Interval		QRS Duration	
(min.)	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE
0	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000
10	0.947	0.054	0.951	0.047	0.939	0.104	0.886	0.093	0.946	0.111	1.262	0.200	1.004	0.039
20	0.904	0.059	0.785	0.063	0.835	0.069	0.662	0.083	0.737	0.102	1.245	0.159	1.026	0.034
30	0.851	0.070	0.764	0.072	0.809	0.094	0.638	0.112	0.680	0.114	1.268	0.160	1.076	0.048
40	0.801	0.057	0.792	0.079	0.811	0.121	0.637	0.118	0.625	0.143	1.418	0.231	1.129	0.056
50	0.749	0.064	0.850	0.040	0.669	0.099	0.583	0.098	0.502	0.149	1.266	0.124	1.069	0.047
60	0.761	0.055	0.800	0.040	0.776	0.095	0.610	0.063	0.515	0.088	1.174	0.113	1.059	0.039
70	0.857	0.019	0.874	0.051	0.981	0.075	0.846	0.055	0.809	0.100	1.098	0.062	1.033	0.071
80	0.843	0.020	0.909	0.043	0.995	0.058	0.911	0.080	0.925	0.154	1.123	0.078	0.956	0.039
90	0.833	0.028	0.892	0.051	1.067	0.071	0.958	0.075	0.969	0.155	1.156	0.085	1.043	0.073
100	0.812	0.026	0.810	0.064	1.056	0.127	0.841	0.114	0.926	0.222	1.215	0.086	1.085	0.074
110	0.795	0.032	0.865	0.057	0.996	0.112	0.858	0.109	0.913	0.205	1.212	0.109	1.019	0.054
120	0.810	0.028	0.898	0.049	0.973	0.103	0.884	0.121	0.765	0.155	1.245	0.059	0.975	0.048

Table B-4. Summary of the physiological parameters for adult chloral hydrate 200 µg/ml experiments, expressed as a fraction of baseline values (N=6).

Time	CF		HR		DP		MVO_2		dP/dT		PR Interval		QRS Duration	
(min.)	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE
0	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000
10	0.877	0.042	0.892	0.035	0.973	0.028	0.868	0.039	0.884	0.056	1.181	0.028	1.064	0.066
20	0.799	0.060	0.617	0.094	0.921	0.057	0.575	0.098	0.758	0.075	1.275	0.067	1.181	0.138
30	0.770	0.037	0.660	0.096	0.937	0.082	0.653	0.115	0.649	0.051	1.238	0.057	1.172	0.087
40	0.769	0.038	0.704	0.103	0.814	0.061	0.597	0.101	0.731	0.061	1.163	0.073	1.138	0.076
50	0.709	0.054	0.661	0.095	0.864	0.076	0.593	0.101	0.558	0.080	1.185	0.086	1.216	0.109
60	0.686	0.060	0.692	0.102	0.926	0.057	0.621	0.084	0.630	0.069	1.156	0.094	1.164	0.119
70	0.811	0.040	0.822	0.045	0.892	0.074	0.723	0.045	0.664	0.057	1.058	0.093	1.167	0.059
80	0.771	0.027	0.811	0.048	0.877	0.075	0.699	0.043	0.596	0.045	1.035	0.080	1.110	0.070
90	0.738	0.033	0.885	0.044	0.869	0.067	0.766	0.064	0.695	0.073	1.158	0.082	1.164	0.071
100	0.726	0.030	0.821	0.058	0.917	0.051	0.745	0.046	0.734	0.080	1.119	0.063	1.093	0.030
110	0.707	0.039	0.805	0.067	0.923	0.047	0.740	0.067	0.710	0.043	1.105	0.059	1.082	0.024
120	0.689	0.041	0.819	0.078	0.848	0.060	0.702	0.091	0.716	0.083	1.106	0.083	1.127	0.033

Table B-5. Summary of the physiological parameters for adult trichloroethanol 25 µg/ml experiments, expressed as a fraction of baseline values (N=6).

Time	CF		HR		DP		MVO_2		dP/dT		PR Interval		QRS Duration	
(min)	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE
0	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000
10	0.933	0.029	0.902	0.031	1.012	0.072	0.920	0.085	0.976	0.095	1.174	0.089	1.139	0.094
20	0.913	0.033	0.919	0.050	0.938	0.115	0.867	0.118	0.834	0.120	1.146	0.050	1.155	0.084
30	0.938	0.051	0.897	0.048	0.996	0.103	0.901	0.119	0.882	0.141	1.197	0.090	1.214	0.068
40	0.884	0.019	0.947	0.071	0.978	0.094	0.935	0.124	0.836	0.176	1.147	0.083	1.186	0.091
50	0.899	0.041	0.863	0.039	1.023	0.090	0.892	0.106	0.786	0.154	1.216	0.068	1.147	0.080
60	0.817	0.044	0.812	0.057	1.048	0.112	0.847	0.107	0.809	0.180	1.450	0.251	1.185	0.104
70	0.914	0.031	0.842	0.028	1.071	0.094	0.904	0.090	0.900	0.172	1.109	0.070	1.142	0.073
80	0.886	0.038	0.800	0.060	1.153	0.185	0.907	0.080	0.871	0.138	1.133	0.063	1.110	0.058
90	0.841	0.045	0.761	0.064	1.148	0.129	0.860	0.092	0.896	0.191	1.051	0.044	1.137	0.059
100	0.848	0.040	0.802	0.070	1.185	0.158	0.941	0.126	1.023	0.230	1.234	0.228	1.106	0.061
110	0.822	0.048	0.719	0.087	1.240	0.167	0.864	0.116	0.915	0.173	1.454	0.434	1.054	0.099
120	0.814	0.046	0.762	0.107	1.242	0.153	0.913	0.116	0.921	0.159	1.467	0.324	1.105	0.049

Table B-6. Summary of the physiological parameters for adult trichloroethanol 50 µg/ml experiments, expressed as a fraction of baseline values (N=7).

Time	CF		HR		DP		MVO_2		dP/dT		PR Interval		QRS Duration	
(min)	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE
0	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000
10	0.985	0.032	0.906	0.033	0.896	0.040	0.820	0.038	0.823	0.077	1.082	0.040	1.066	0.038
20	0.987	0.037	0.864	0.090	0.938	0.040	0.808	0.074	0.690	0.100	1.178	0.099	0.985	0.020
30	0.923	0.039	0.877	0.096	0.898	0.050	0.778	0.062	0.643	0.116	1.158	0.131	1.043	0.038
40	0.887	0.060	0.868	0.101	0.833	0.060	0.709	0.058	0.597	0.075	1.102	0.080	1.062	0.036
50	0.872	0.049	0.962	0.114	0.779	0.096	0.714	0.080	0.612	0.114	1.072	0.109	1.026	0.034
60	0.849	0.060	1.006	0.092	0.706	0.076	0.705	0.077	0.538	0.115	1.135	0.141	1.032	0.026
70	0.926	0.058	0.974	0.074	0.843	0.065	0.832	0.087	0.683	0.107	1.054	0.080	1.033	0.059
80	0.860	0.063	0.849	0.103	0.856	0.045	0.737	0.102	0.738	0.153	1.128	0.090	1.071	0.028
90	0.845	0.064	0.817	0.099	0.794	0.038	0.653	0.081	0.651	0.124	1.084	0.087	1.016	0.027
100	0.830	0.074	0.853	0.114	0.800	0.054	0.675	0.086	0.591	0.120	1.004	0.098	1.034	0.068
110	0.821	0.081	0.863	0.116	0.804	0.052	0.682	0.079	0.641	0.091	1.097	0.149	1.041	0.066
120	0.785	0.082	0.865	0.116	0.793	0.050	0.678	0.088	0.640	0.110	1.128	0.158	1.035	0.075

Table B-7. Summary of the physiological parameters for adult trichloroethanol 100 µg/ml experiments, expressed as a fraction of baseline values (N=7).

Time	CF		HR		DP		MVO_2		dP/dT		PR Interval		QRS Duration	
(min.)	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE
0	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000
10	0.915	0.046	0.877	0.051	1.019	0.106	0.853	0.077	0.874	0.075	1.438	0.236	1.010	0.047
20	0.854	0.100	0.758	0.128	0.911	0.143	0.608	0.075	0.579	0.098	1.393	0.163	1.124	0.083
30	0.853	0.081	0.912	0.211	0.948	0.197	0.738	0.107	0.658	0.160	1.618	0.273	1.091	0.041
40	0.856	0.089	0.871	0.156	0.961	0.174	0.757	0.105	0.655	0.132	1.302	0.115	1.088	0.043
50	0.787	0.088	0.789	0.069	0.946	0.189	0.696	0.104	0.570	0.152	1.390	0.143	1.187	0.099
60	0.805	0.081	0.839	0.068	0.920	0.179	0.721	0.096	0.563	0.132	1.328	0.206	1.080	0.099
70	0.924	0.057	0.975	0.142	1.014	0.167	0.878	0.073	0.764	0.128	1.331	0.174	1.174	0.085
80	0.830	0.048	0.903	0.041	1.077	0.167	0.937	0.103	0.908	0.148	1.087	0.057	1.085	0.047
90	0.816	0.069	0.888	0.052	1.043	0.136	0.898	0.094	0.925	0.161	1.200	0.090	1.015	0.029
100	0.807	0.079	0.892	0.039	1.083	0.113	0.951	0.083	0.916	0.147	1.141	0.087	1.013	0.033
110	0.749	0.056	0.892	0.049	1.015	0.100	0.884	0.059	0.898	0.100	1.255	0.062	1.101	0.053
120	0.740	0.064	0.848	0.052	1.025	0.105	0.845	0.052	0.860	0.099	1.200	0.088	1.094	0.039

Table B-8. Summary of the physiological parameters for adult TCE 150 µg/ml experiments, expressed as a fraction of baseline values (N=6).

Time	CF		HR		DP		MVO_2		dP/dT		PR Interval		QRS Duration	
(min.)	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE
0	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000
10	0.964	0.022	1.016	0.034	0.867	0.048	0.881	0.057	0.788	0.100	1.102	0.043	1.005	0.073
20	0.968	0.036	0.745	0.109	0.904	0.163	0.658	0.125	0.601	0.124	1.032	0.057	1.056	0.043
30	0.905	0.031	0.920	0.071	0.886	0.080	0.804	0.068	0.577	0.107	1.041	0.073	1.079	0.090
40	0.874	0.032	0.893	0.056	0.872	0.049	0.775	0.052	0.539	0.095	1.194	0.055	1.089	0.075
50	0.857	0.043	0.707	0.071	0.988	0.116	0.677	0.077	0.505	0.105	1.383	0.179	1.097	0.094
60	0.838	0.038	0.715	0.063	0.943	0.105	0.659	0.071	0.535	0.096	1.388	0.215	1.057	0.087
70	0.880	0.059	0.828	0.036	1.199	0.197	1.001	0.173	0.804	0.194	1.431	0.215	1.096	0.121
80	0.825	0.055	0.599	0.068	1.262	0.088	0.735	0.089	0.799	0.146	1.405	0.219	1.035	0.072
90	0.863	0.058	0.648	0.092	1.323	0.055	0.852	0.123	0.871	0.053	1.350	0.242	0.943	0.020
100	0.816	0.067	0.625	0.096	1.353	0.129	0.874	0.203	0.950	0.167	1.399	0.220	0.975	0.035
110	0.784	0.044	0.651	0.103	1.274	0.054	0.817	0.113	0.959	0.155	1.280	0.186	0.982	0.042
120	0.788	0.046	0.638	0.077	1.350	0.116	0.886	0.178	0.890	0.170	1.283	0.219	0.982	0.036

Table B-9. Summary of the physiological parameters for adult trichloroacetic acid 25 µg/ml experiments, expressed as a fraction of baseline values (N=6).

Time	CF		HR		DP		MVO_2		dP/dT		PR Interval		QRS Duration	
(min.)	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE
0	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000
10	0.937	0.045	1.008	0.041	0.924	0.057	0.923	0.031	0.904	0.117	1.183	0.095	0.961	0.019
20	0.924	0.077	0.876	0.054	0.913	0.091	0.793	0.086	0.809	0.150	1.291	0.087	1.080	0.066
30	0.879	0.060	0.845	0.067	0.926	0.081	0.790	0.104	0.895	0.192	1.306	0.104	1.055	0.037
40	0.842	0.062	0.877	0.053	0.968	0.058	0.855	0.078	0.962	0.191	1.342	0.117	1.253	0.089
50	0.790	0.074	0.866	0.069	0.880	0.067	0.764	0.087	0.754	0.193	1.361	0.103	1.424	0.157
60	0.801	0.061	0.889	0.068	0.913	0.051	0.818	0.087	0.854	0.190	1.357	0.158	1.277	0.103
70	0.959	0.064	0.974	0.035	0.987	0.036	0.962	0.045	0.855	0.125	1.332	0.169	1.192	0.117
80	0.932	0.059	0.967	0.068	0.995	0.070	0.962	0.086	1.080	0.335	1.342	0.197	1.101	0.066
90	0.897	0.052	0.951	0.065	1.003	0.093	0.937	0.079	1.000	0.398	1.343	0.196	1.095	0.077
100	0.861	0.055	0.901	0.074	1.010	0.121	0.909	0.122	0.783	0.172	1.324	0.233	1.160	0.114
110	0.826	0.055	0.911	0.071	0.996	0.103	0.905	0.110	0.689	0.113	1.318	0.246	1.163	0.053
120	0.821	0.053	0.908	0.081	1.032	0.109	0.915	0.093	0.709	0.126	1.285	0.187	1.139	0.048

Table B-10. Summary of the physiological parameters for adult trichloroacetic acid 50 µg/ml experiments, expressed as a fraction of baseline values (N=7).

Time	CF		HR		DP		MVO_2		dP/dT		PR Interval		QRS Duration	
(min.)	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE
0	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000
10	0.860	0.029	0.890	0.031	1.080	0.109	0.996	0.110	1.042	0.180	1.020	0.043	1.082	0.054
20	0.815	0.052	0.911	0.040	0.978	0.114	0.891	0.105	0.919	0.116	1.203	0.083	1.177	0.096
30	0.816	0.062	0.871	0.049	0.933	0.117	0.815	0.100	0.817	0.099	1.567	0.190	1.477	0.256
40	0.769	0.043	0.817	0.060	0.875	0.110	0.714	0.099	0.701	0.110	1.683	0.172	1.191	0.094
50	0.733	0.044	0.789	0.084	0.890	0.096	0.691	0.083	0.697	0.080	1.709	0.204	1.144	0.121
60	0.724	0.046	0.772	0.053	0.822	0.065	0.645	0.075	0.659	0.111	1.956	0.230	1.237	0.143
70	0.854	0.036	0.781	0.117	0.864	0.084	0.677	0.108	0.632	0.108	1.725	0.331	1.228	0.113
80	0.758	0.033	0.752	0.082	0.830	0.071	0.635	0.086	0.601	0.089	2.156	0.321	1.296	0.111
90	0.727	0.046	0.758	0.074	0.746	0.127	0.659	0.103	0.565	0.126	1.959	0.213	1.211	0.116
100	0.750	0.031	0.867	0.079	0.795	0.094	0.696	0.113	0.599	0.092	1.816	0.222	1.265	0.109
110	0.736	0.048	0.906	0.085	0.796	0.078	0.724	0.107	0.616	0.110	1.708	0.218	1.194	0.092
120	0.696	0.041	0.863	0.082	0.818	0.077	0.715	0.105	0.587	0.101	1.712	0.231	1.123	0.097

Table B-11. Summary of the physiological parameters for adult trichloroacetic acid 100 µg/ml experiments, expressed as a fraction of baseline values (N=6).

Time	CF		HR		DP		MVO_2		dP/dT		PR Interval		QRS Duration	
(min.)	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE
0	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000
10	0.843	0.031	0.892	0.053	0.917	0.052	0.821	0.071	0.972	0.070	1.096	0.029	1.085	0.058
20	0.829	0.024	0.809	0.039	0.952	0.092	0.771	0.087	0.963	0.118	1.603	0.267	1.149	0.060
30	0.800	0.037	0.718	0.085	0.947	0.097	0.705	0.131	0.828	0.150	1.453	0.212	1.201	0.138
40	0.737	0.042	0.691	0.105	0.956	0.073	0.658	0.113	0.808	0.101	1.598	0.234	1.149	0.110
50	0.801	0.059	0.714	0.115	0.889	0.087	0.624	0.108	0.780	0.089	1.598	0.190	1.231	0.082
60	0.756	0.058	0.809	0.125	0.902	0.074	0.712	0.108	0.819	0.106	1.639	0.230	1.188	0.076
70	0.869	0.078	0.850	0.076	0.995	0.060	0.832	0.072	0.999	0.105	1.615	0.255	1.098	0.069
80	0.787	0.071	0.921	0.061	0.894	0.027	0.820	0.051	0.819	0.078	1.330	0.231	1.229	0.159
90	0.837	0.034	0.951	0.049	0.835	0.040	0.787	0.031	0.756	0.045	1.242	0.219	1.172	0.116
100	0.818	0.035	0.981	0.081	0.839	0.050	0.813	0.052	0.729	0.051	1.100	0.226	1.182	0.170
110	0.771	0.030	0.936	0.037	0.843	0.064	0.779	0.043	0.698	0.046	1.266	0.102	1.294	0.164
120	0.803	0.043	0.989	0.094	0.848	0.054	0.818	0.037	0.665	0.060	1.120	0.123	1.160	0.106

Table B-12. Summary of the physiological parameters for adult trichloroacetic acid 150 µg/ml experiments, expressed as a fraction of baseline values (N=6).

Time	CF		HR		DP		MVO_2		dP/dT		PR Interval		QRS Duration	
(min.)	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE
0	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000
10	0.918	0.045	0.970	0.024	0.927	0.039	0.911	0.052	0.965	0.086	1.005	0.027	0.949	0.022
20	0.874	0.047	0.656	0.116	0.852	0.094	0.599	0.147	0.655	0.127	1.222	0.122	1.063	0.065
30	0.846	0.052	0.732	0.094	0.938	0.090	0.686	0.095	0.646	0.107	1.408	0.110	1.113	0.111
40	0.804	0.077	0.710	0.072	0.913	0.076	0.668	0.113	0.743	0.074	1.501	0.124	1.083	0.069
50	0.775	0.101	0.843	0.042	0.924	0.051	0.782	0.053	0.744	0.088	1.461	0.190	1.048	0.041
60	0.831	0.072	0.731	0.101	0.809	0.159	0.651	0.181	0.654	0.213	1.437	0.204	1.146	0.075
70	0.846	0.059	0.902	0.047	0.917	0.123	0.812	0.078	0.709	0.176	1.287	0.266	1.129	0.087
80	0.767	0.049	0.962	0.162	0.993	0.110	0.915	0.067	0.796	0.154	1.332	0.252	1.123	0.053
90	0.793	0.070	0.861	0.078	1.098	0.148	0.983	0.148	0.924	0.309	1.259	0.263	1.052	0.080
100	0.749	0.085	0.836	0.064	0.913	0.185	0.781	0.166	0.751	0.251	1.391	0.265	1.024	0.039
110	0.726	0.083	0.861	0.076	0.898	0.126	0.779	0.128	0.804	0.252	1.552	0.353	1.073	0.050
120	0.699	0.072	0.827	0.064	0.813	0.074	0.681	0.085	0.515	0.067	1.397	0.254	1.085	0.054

Table B-13. Maximal adult pharmacodynamic effects for control, chloral hydrate (50, 100 and 200 µg/ml), trichloroethanol (25, 50, 100 and 150 µg/ml) and trichloroacetic acid (25, 50, 100 and 150 µg/ml) experiments.[§]

Experiment		CF Ave. SE	HR Ave. SE	DP Ave. SE	MVO_2 Ave. SE	dP/dT Ave. SE	PR Ave. SE	QRS Ave. SE
CONTROL		0.924 0.015	0.981 0.014	1.018 0.018	1.010 0.011	1.042 0.020	1.013 0.008	0.979 0.015
CH	50	0.759 0.050	0.794 0.035	0.715 0.036	0.623 0.058	0.502 0.059	1.362 0.134	1.229 0.084
	100	0.718 0.060	0.685 0.065	0.648 0.095	0.518 0.077	0.459 0.096	1.427 0.213	1.202 0.042
	200	0.685 0.059	0.535 0.074	0.781 0.059	0.516 0.092	0.541 0.077	1.336 0.038	1.269 0.124
TCE	25	0.808 0.039	0.783 0.047	0.882 0.100	0.773 0.101	0.708 0.134	1.562 0.233	1.321 0.080
	50	0.829 0.052	0.815 0.081	0.668 0.076	0.640 0.075	0.534 0.097	1.299 0.096	1.102 0.036
	100	0.726 0.072	0.641 0.090	0.681 0.112	0.527 0.068	0.483 0.116	1.753 0.247	1.263 0.094
	150	0.809 0.029	0.609 0.069	0.808 0.111	0.576 0.128	0.428 0.083	1.424 0.206	1.179 0.083
TCA	25	0.759 0.053	0.786 0.063	0.804 0.072	0.672 0.067	0.618 0.146	1.444 0.139	1.466 0.139
	50	0.674 0.048	0.682 0.052	0.716 0.060	0.515 0.060	0.513 0.063	1.847 0.206	1.591 0.255
	100	0.688 0.033	0.620 0.094	0.737 0.086	0.587 0.110	0.685 0.100	1.786 0.254	1.306 0.119
	150	0.634 0.088	0.565 0.087	0.611 0.118	0.417 0.135	0.404 0.120	1.827 0.325	1.202 0.107

[§] Maximal effects were determined in the treatment phase of the protocol only, and are expressed fractions of the respective baseline values.

APPENDIX C

- Representative Neonatal Physiologic Tracings
- Summaries of Neonatal Physiologic Parameters
- Maximal Neonatal Pharmacodynamic Effects

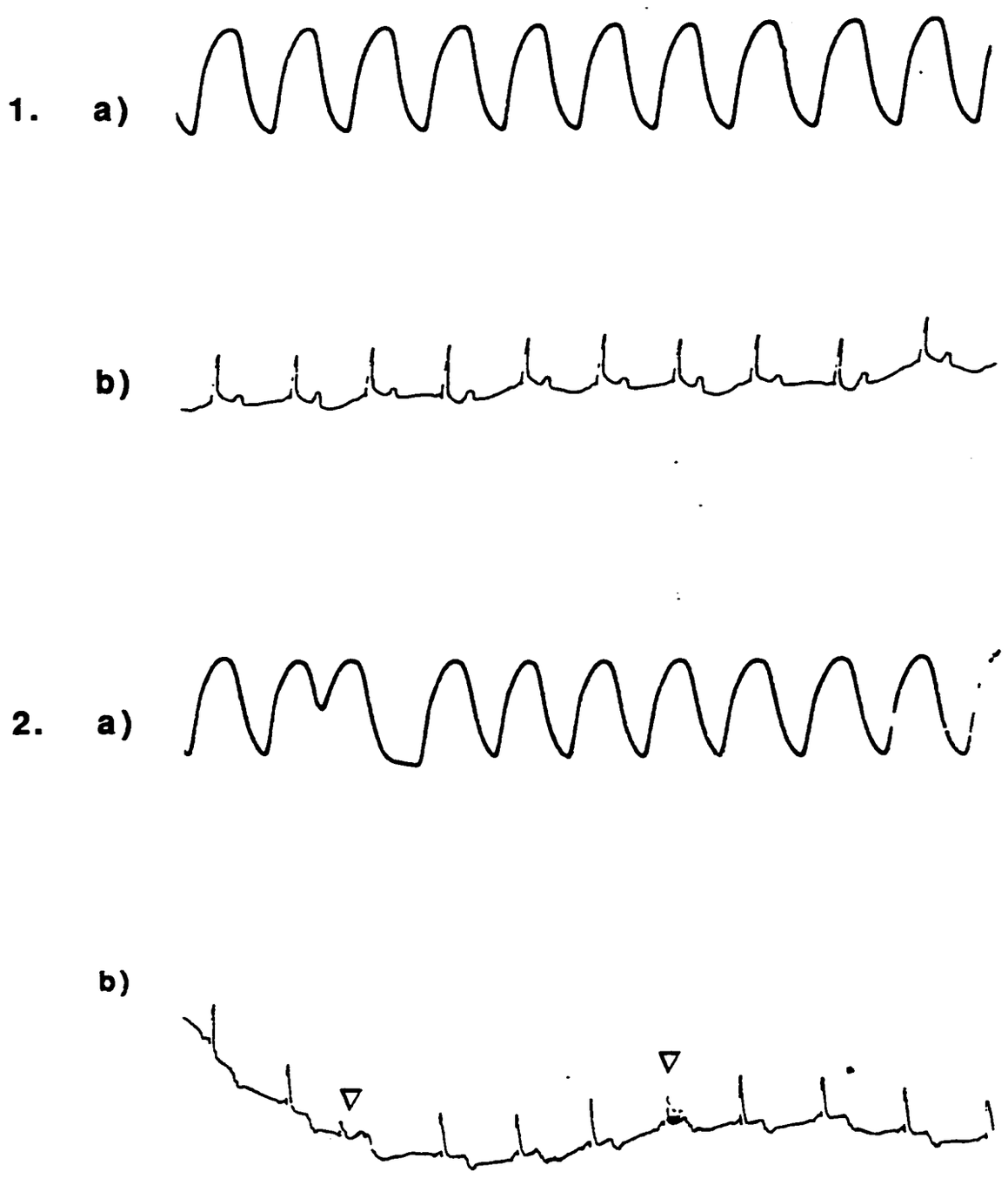


Figure C-1. Simultaneous left ventricular pressure (a) and lead I ECG (b) tracings of a representative chloral hydrate treated (100 μg/ml) neonatal rabbit heart (sensitivity=100 mV/mm; chart speed= 25 mm/s). 1(a) and (b) illustrate normal pressure and ECG indices at T=0 of the protocol. 2(b) Note the occurrence of premature ventricular contractions at T=25 min. of the protocol.

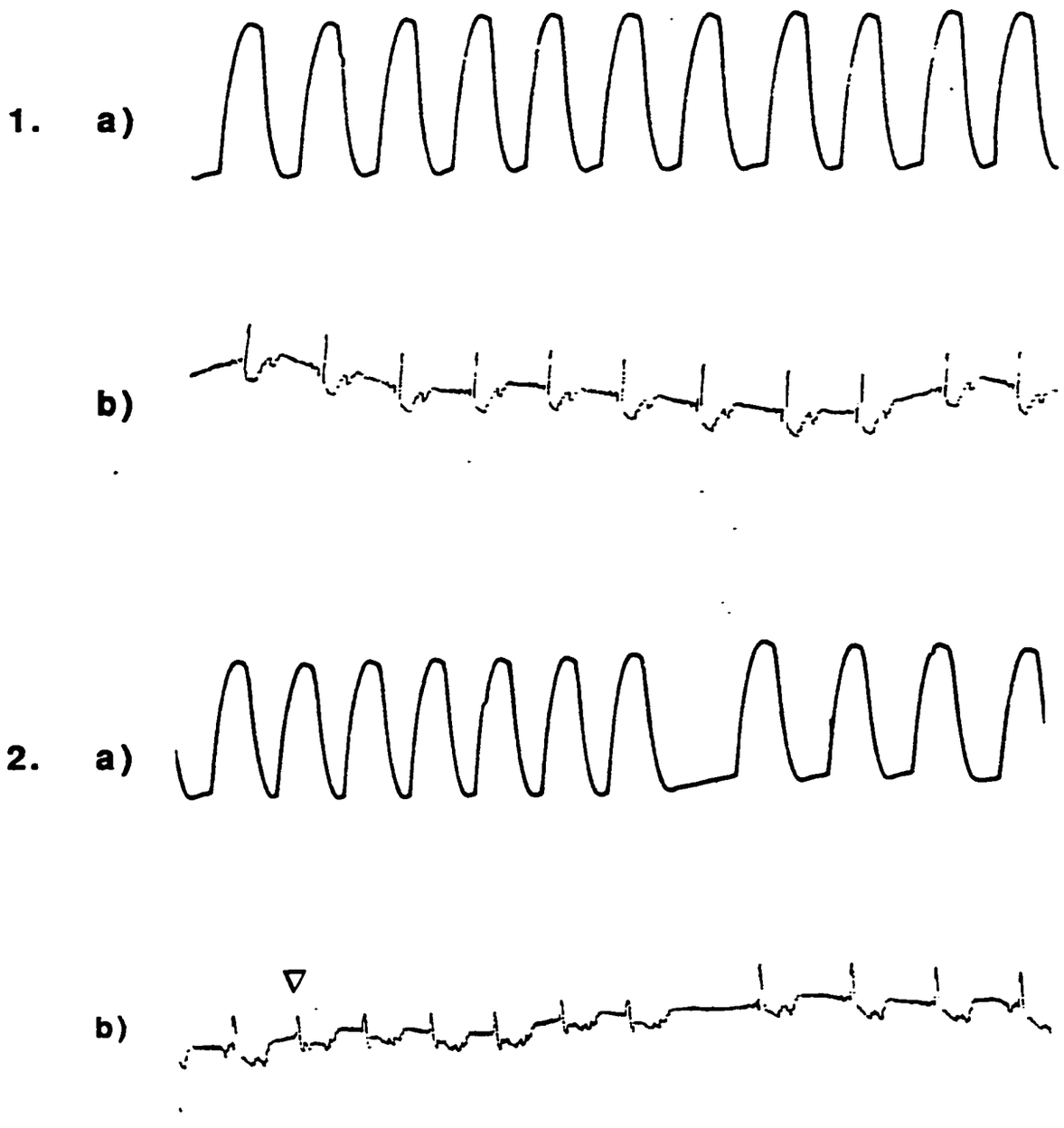


Figure C-2. Simultaneous left ventricular pressure (a) and lead I ECG (b) tracings of a representative chloral hydrate treated (200 µg/ml) neonatal rabbit heart (sensitivity=100 mV/mm; chart speed= 25 mm/s). 1(a) and (b) illustrate normal pressure and ECG indices at T=0 of the protocol (slight ST segment depression and T wave inversion are normal for this preparation). 2(b) Note the development of ventricular tachycardia at T=60 min. of the protocol.

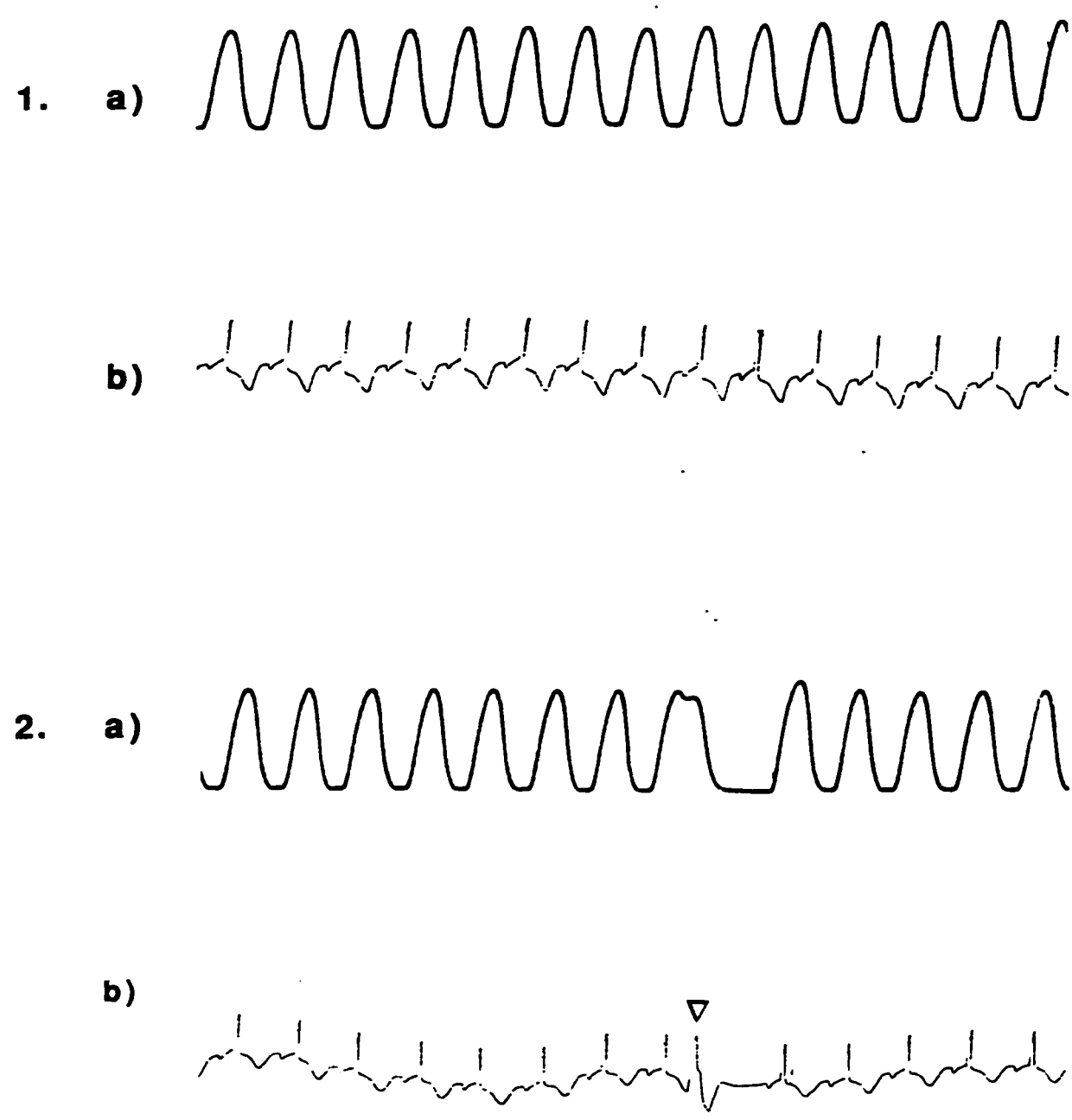


Figure C-3. Simultaneous left ventricular pressure (a) and lead I ECG (b) tracings of a representative trichloroethanol treated (150 µg/ml) neonatal rabbit heart (sensitivity=100 mV/mm; chart speed= 25 mm/s). 1(a) and (b) illustrate normal pressure and ECG indices at T=0 of the protocol (slight ST segment depression and T wave inversion are normal for this preparation). 2(b) Note the presence of an ectopic beat at T=30 min. of the protocol.

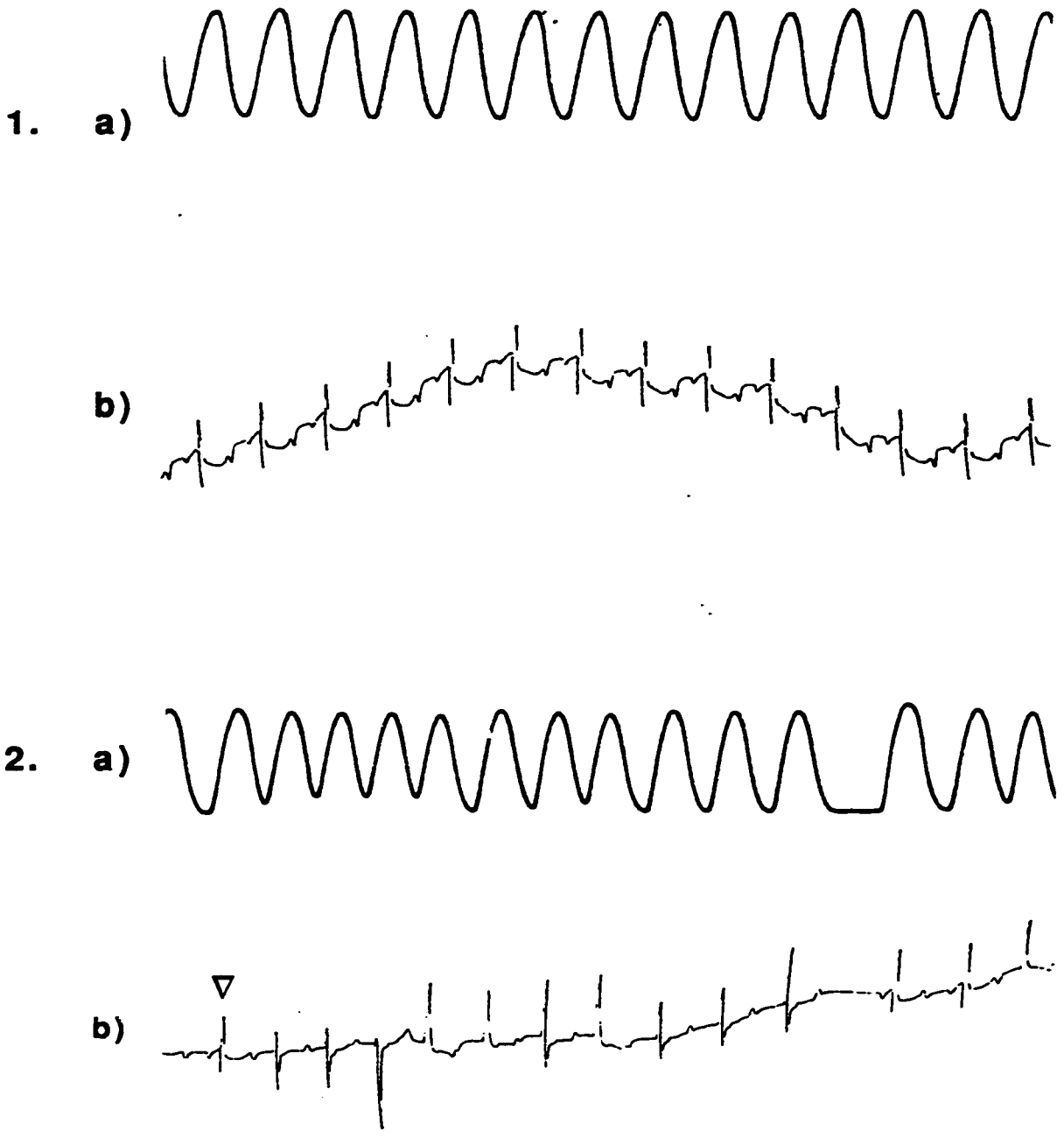


Figure C-4. Simultaneous left ventricular pressure (a) and lead I ECG (b) tracings of a representative trichoroacetic acid treated (25 µg/ml) neonatal rabbit heart (sensitivity=100 mV/mm; chart speed= 25 mm/s). 1(a) and (b) illustrate normal pressure and ECG indices at T=0 of the protocol (slight ST segment depression and T wave inversion are normal for this preparation). 2(b) Note the development of ectopic beats at T=50 min. of the protocol.

Table C-1. Summary of the physiological parameters for neonatal control experiments, expressed as a fraction of baseline values (N=12).

Time (min.)	CF		HR		DP		MVO_2		dP/dT		PR Interval		QRS Duration	
	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE
0	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000
10	0.986	0.005	0.987	0.022	0.992	0.017	0.978	0.023	0.985	0.008	0.978	0.028	1.008	0.036
20	0.990	0.015	0.979	0.027	1.009	0.022	0.991	0.044	1.008	0.013	0.998	0.034	0.957	0.031
30	0.968	0.018	0.993	0.024	0.988	0.023	0.979	0.023	0.994	0.012	1.010	0.043	0.998	0.021
40	0.954	0.016	0.997	0.021	1.020	0.032	1.017	0.032	0.999	0.014	0.999	0.039	0.968	0.014
50	0.973	0.018	1.003	0.020	1.014	0.039	1.015	0.041	1.003	0.014	1.022	0.060	0.950	0.016
60	0.945	0.014	0.971	0.023	1.009	0.044	0.992	0.043	0.989	0.018	0.997	0.040	1.012	0.029
70	0.944	0.016	0.978	0.030	1.041	0.053	1.012	0.044	1.027	0.022	1.012	0.037	0.989	0.041
80	0.940	0.020	0.972	0.028	1.018	0.041	0.991	0.048	1.001	0.017	1.021	0.044	0.977	0.044
90	0.934	0.018	0.969	0.028	1.016	0.048	0.985	0.054	0.986	0.020	1.018	0.039	0.959	0.029
100	0.914	0.020	0.958	0.028	1.016	0.034	0.974	0.042	0.986	0.016	1.064	0.051	0.933	0.028
110	0.904	0.026	0.942	0.031	1.004	0.057	0.942	0.048	0.953	0.019	1.039	0.039	0.939	0.031
120	0.929	0.027	0.973	0.040	1.006	0.041	0.974	0.043	0.968	0.018	1.048	0.034	0.966	0.045

Table C-2. Summary of the physiological parameters for neonatal chloral hydrate 50 µg/ml experiments, expressed as a fraction of baseline values (N=6).

Time	CF		HR		DP		MVO_2		dP/dT		PR Interval		QRS Duration	
(min.)	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE
0	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000
10	0.849	0.053	0.946	0.011	0.909	0.052	0.859	0.048	0.934	0.039	1.041	0.026	1.034	0.023
20	0.739	0.044	0.969	0.031	1.087	0.143	1.060	0.155	1.126	0.174	1.055	0.032	1.063	0.030
30	0.748	0.039	0.914	0.039	1.047	0.139	0.952	0.128	1.090	0.167	1.030	0.022	0.960	0.069
40	0.682	0.008	0.900	0.032	1.048	0.160	0.941	0.152	1.057	0.176	1.133	0.013	1.009	0.029
50	0.700	0.033	0.871	0.030	1.005	0.166	0.882	0.166	1.001	0.202	1.147	0.039	1.030	0.041
60	0.715	0.045	0.895	0.029	0.972	0.179	0.866	0.164	0.979	0.182	1.156	0.036	1.006	0.031
70	0.964	0.037	1.013	0.041	1.268	0.171	1.279	0.172	1.253	0.170	0.937	0.043	0.973	0.035
80	0.926	0.044	1.037	0.044	1.244	0.128	1.269	0.129	1.251	0.135	1.012	0.043	0.988	0.038
90	0.919	0.037	1.085	0.058	1.246	0.192	1.362	0.255	1.292	0.159	0.973	0.028	0.972	0.027
100	0.883	0.048	1.055	0.060	1.233	0.187	1.312	0.238	1.270	0.179	1.005	0.031	1.014	0.042
110	0.888	0.037	1.056	0.058	1.219	0.162	1.285	0.195	1.226	0.152	1.002	0.033	1.067	0.033
120	0.828	0.039	1.066	0.064	1.149	0.144	1.219	0.175	1.191	0.132	1.026	0.034	1.036	0.038

Table C-3. Summary of the physiological parameters for neonatal chloral hydrate 100 µg/ml experiments, expressed as a fraction of baseline values (N=6).

110	0.888	0.037	1.056	0.058	1.219	0.162	1.285	0.195	1.226	0.152	1.002	0.033	1.067	0.033
120	0.828	0.039	1.066	0.064	1.149	0.144	1.219	0.175	1.191	0.132	1.026	0.034	1.036	0.038

Table C-3. Summary of the physiological parameters for neonatal chloral hydrate 100 µg/ml experiments, expressed as a fraction of baseline values (N=6).

Time (min.)	CF		HR		DP		MVO_2		dP/dT		PR Interval		QRS Duration	
	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE
0	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000
10	0.921	0.027	0.978	0.023	0.984	0.063	0.999	0.050	1.059	0.038	0.935	0.048	1.011	0.035
20	0.921	0.077	0.939	0.058	0.887	0.095	0.860	0.094	0.940	0.064	1.035	0.064	1.069	0.033
30	0.882	0.049	0.908	0.060	0.862	0.091	0.813	0.096	0.926	0.069	1.005	0.045	1.054	0.029
40	0.849	0.024	0.917	0.053	0.921	0.090	0.886	0.110	1.056	0.050	0.963	0.059	1.022	0.014
50	0.840	0.025	0.930	0.050	0.854	0.080	0.833	0.098	0.995	0.078	0.953	0.063	1.073	0.037
60	0.848	0.049	0.908	0.025	0.809	0.092	0.766	0.088	0.905	0.073	0.961	0.048	1.051	0.034
70	0.906	0.036	1.004	0.053	0.775	0.095	0.816	0.112	1.030	0.071	0.940	0.063	1.058	0.053
80	0.868	0.043	0.958	0.051	0.754	0.087	0.746	0.083	0.993	0.083	0.944	0.046	1.079	0.046
90	0.816	0.034	0.996	0.047	0.777	0.100	0.808	0.110	1.009	0.092	0.934	0.058	1.039	0.035
100	0.782	0.028	0.972	0.043	0.810	0.081	0.813	0.081	1.044	0.091	0.947	0.071	1.037	0.021
110	0.769	0.036	0.972	0.037	0.775	0.075	0.784	0.078	1.035	0.088	0.967	0.059	1.086	0.039
120	0.788	0.039	0.982	0.044	0.755	0.071	0.772	0.078	0.981	0.075	0.970	0.048	1.048	0.036

Table C-4. Summary of the physiological parameters for neonatal chloral hydrate 200 µg/ml experiments, expressed as a fraction of baseline values (N=7).

Time	CF		HR		DP		MVO_2		dP/dT		PR Interval		QRS Duration	
(min.)	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE	Ave	SE	Ave	SE	Ave	SE
0	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000
10	0.999	0.023	0.984	0.012	1.024	0.062	1.006	0.058	1.072	0.114	0.992	0.044	0.990	0.032
20	0.942	0.031	0.838	0.036	1.209	0.165	1.011	0.139	1.154	0.162	1.012	0.043	1.009	0.017
30	0.943	0.023	0.716	0.063	1.159	0.183	0.809	0.117	1.005	0.129	1.071	0.034	0.962	0.037
40	0.893	0.022	0.740	0.058	1.072	0.188	0.804	0.146	1.009	0.150	1.034	0.059	0.982	0.044
50	0.906	0.024	0.743	0.068	1.023	0.182	0.738	0.109	0.978	0.136	1.259	0.258	0.939	0.023
60	0.888	0.022	0.746	0.068	1.028	0.195	0.734	0.101	0.986	0.124	1.055	0.034	0.921	0.036
70	1.013	0.038	0.796	0.065	1.025	0.211	0.788	0.128	0.986	0.167	1.062	0.022	0.993	0.047
80	0.975	0.035	0.873	0.034	1.002	0.202	0.858	0.149	1.028	0.185	1.035	0.028	0.964	0.045
90	0.943	0.039	0.851	0.057	0.935	0.177	0.745	0.094	0.832	0.116	1.054	0.021	1.027	0.049
100	0.943	0.035	0.910	0.042	0.906	0.182	0.782	0.117	0.811	0.101	1.087	0.029	0.964	0.026
110	0.928	0.040	0.897	0.034	0.871	0.176	0.748	0.118	0.846	0.113	1.089	0.020	0.930	0.019
120	0.924	0.042	0.901	0.025	0.903	0.156	0.757	0.132	0.841	0.122	1.096	0.043	0.991	0.038

Table C-5. Summary of the physiological parameters for neonatal trichloroethanol 25 µg/ml experiments, expressed as a fraction of baseline values (N=6).

Time	CF		HR		DP		MVO_2		dP/dT		PR Interval		QRS Duration	
(min.)	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE
0	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000
10	0.937	0.007	0.985	0.037	1.057	0.056	1.043	0.064	0.980	0.082	1.061	0.038	0.985	0.024
20	0.906	0.022	0.968	0.051	1.147	0.100	1.097	0.072	1.152	0.062	1.006	0.056	0.936	0.025
30	0.901	0.022	0.940	0.069	1.052	0.068	0.979	0.075	1.063	0.068	1.027	0.038	1.021	0.053
40	0.868	0.022	0.947	0.065	1.072	0.083	0.997	0.054	1.041	0.078	0.967	0.056	1.016	0.052
50	0.853	0.022	0.948	0.057	1.015	0.090	0.942	0.043	0.973	0.103	1.011	0.031	0.991	0.054
60	0.827	0.022	0.891	0.049	0.968	0.093	0.849	0.062	0.914	0.065	1.068	0.040	0.962	0.019
70	0.969	0.024	0.977	0.056	1.100	0.097	1.067	0.099	1.003	0.073	1.045	0.031	0.931	0.025
80	0.942	0.042	0.987	0.044	1.126	0.102	1.104	0.091	1.007	0.091	1.036	0.020	0.955	0.035
90	0.914	0.020	1.006	0.033	1.048	0.146	1.043	0.129	0.998	0.126	1.062	0.041	0.933	0.029
100	0.933	0.028	0.945	0.046	1.135	0.123	1.051	0.076	1.027	0.109	1.059	0.028	0.976	0.061
110	0.897	0.037	0.913	0.027	1.077	0.130	0.973	0.102	0.956	0.107	1.056	0.041	1.006	0.040
120	0.892	0.033	0.910	0.050	1.049	0.114	0.936	0.088	1.046	0.165	1.095	0.030	0.955	0.037

Table C-6. Summary of the physiological parameters for neonatal trichloroethanol 50 µg/ml experiments, expressed as a fraction of baseline values (N=6).

Time	CF		HR		DP		MVO_2		dP/dT		PR Interval		QRS Duration	
(min.)	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE
0	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000
10	0.985	0.027	0.995	0.008	1.121	0.053	1.115	0.052	1.093	0.053	1.011	0.026	1.000	0.016
20	0.959	0.020	0.869	0.045	1.062	0.109	0.937	0.124	1.029	0.097	1.075	0.061	0.902	0.043
30	0.944	0.027	0.949	0.040	1.056	0.136	1.002	0.127	1.306	0.274	1.075	0.044	1.043	0.035
40	0.921	0.029	0.904	0.060	1.127	0.132	1.028	0.140	1.179	0.160	1.066	0.065	1.029	0.052
50	0.894	0.031	0.995	0.050	0.970	0.105	0.962	0.100	1.122	0.161	1.052	0.021	1.005	0.022
60	0.900	0.024	0.967	0.054	1.044	0.089	1.001	0.082	1.068	0.133	0.997	0.051	0.984	0.035
70	1.017	0.031	1.030	0.062	1.173	0.102	1.202	0.109	1.422	0.230	0.995	0.027	0.991	0.040
80	0.991	0.024	0.987	0.033	1.162	0.075	1.140	0.063	1.449	0.260	0.948	0.084	1.016	0.022
90	0.975	0.026	0.999	0.047	1.151	0.129	1.148	0.133	1.353	0.214	0.989	0.077	0.998	0.070
100	0.949	0.021	0.989	0.053	1.171	0.122	1.151	0.124	1.356	0.213	0.997	0.024	1.063	0.046
110	0.920	0.022	0.973	0.058	1.146	0.149	1.112	0.150	1.263	0.214	1.048	0.027	1.042	0.039
120	0.887	0.022	0.988	0.050	1.107	0.151	1.090	0.147	1.144	0.153	1.068	0.009	1.053	0.030

Table C-7. Summary of the physiological parameters for neonatal trichloroethanol 100 µg/ml experiments, expressed as a fraction of baseline values (N=6).

Time	CF		HR		DP		MVO_2		dP/dT		PR Interval		QRS Duration	
(min.)	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE
0	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000
10	0.984	0.028	0.981	0.028	1.098	0.034	1.080	0.051	0.933	0.071	1.004	0.023	1.091	0.018
20	0.967	0.015	0.903	0.065	0.904	0.150	0.801	0.142	0.721	0.130	1.086	0.107	0.944	0.035
30	0.958	0.019	0.815	0.087	1.087	0.211	0.844	0.177	0.856	0.154	1.129	0.138	1.034	0.038
40	0.926	0.029	0.792	0.095	1.255	0.262	0.943	0.225	0.852	0.153	1.232	0.248	1.069	0.063
50	0.918	0.021	0.906	0.057	1.155	0.235	1.034	0.226	0.828	0.150	1.116	0.117	1.029	0.041
60	0.896	0.015	0.896	0.050	1.255	0.254	1.099	0.216	0.814	0.110	1.131	0.120	1.044	0.033
70	0.995	0.018	0.978	0.050	1.331	0.291	1.254	0.263	0.963	0.111	1.185	0.138	1.094	0.033
80	0.990	0.025	0.876	0.053	1.409	0.248	1.201	0.197	0.851	0.091	1.122	0.088	1.056	0.030
90	0.945	0.034	0.900	0.025	1.198	0.171	1.072	0.154	0.798	0.110	1.143	0.111	1.054	0.030
100	0.935	0.022	0.921	0.026	1.221	0.182	1.129	0.180	0.839	0.110	1.132	0.108	1.078	0.027
110	0.915	0.031	0.934	0.020	1.289	0.213	1.206	0.211	0.836	0.112	1.128	0.101	1.080	0.049
120	0.895	0.036	0.875	0.014	1.018	0.117	0.896	0.111	0.774	0.092	1.195	0.112	1.075	0.043

Table C-8. Summary of the physiological parameters for neonatal trichloroethanol 150 µg/ml experiments, expressed as a fraction of baseline values (N=7).

Time	CF		HR		DP		MVO_2		dP/dT		PR Interval		QRS Duration	
(min.)	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE	Ave	SE	Ave	SE	Ave	SE
0	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000
10	0.985	0.024	0.973	0.022	1.043	0.041	1.013	0.036	0.994	0.046	1.001	0.015	1.030	0.029
20	0.924	0.008	0.868	0.035	0.906	0.030	0.784	0.029	1.029	0.056	1.015	0.026	1.023	0.038
30	0.915	0.006	0.912	0.037	0.912	0.028	0.829	0.032	0.986	0.044	1.021	0.019	1.040	0.050
40	0.898	0.004	0.904	0.046	0.891	0.040	0.802	0.043	0.931	0.040	1.039	0.012	1.019	0.051
50	0.853	0.047	0.935	0.051	0.948	0.046	0.894	0.080	0.956	0.044	1.044	0.022	1.032	0.051
60	0.877	0.019	0.931	0.033	0.903	0.073	0.842	0.074	0.904	0.053	1.106	0.012	1.038	0.063
70	0.962	0.026	0.947	0.030	0.978	0.087	0.931	0.098	1.009	0.107	1.093	0.017	1.064	0.037
80	0.911	0.020	0.922	0.052	0.966	0.067	0.893	0.081	0.977	0.088	1.073	0.033	1.072	0.061
90	0.911	0.024	0.979	0.036	1.011	0.083	0.995	0.095	1.053	0.067	1.056	0.031	0.991	0.027
100	0.898	0.023	0.997	0.029	0.980	0.060	0.979	0.067	1.068	0.074	1.097	0.028	0.989	0.034
110	0.873	0.021	1.047	0.058	0.958	0.074	1.011	0.107	1.043	0.108	1.113	0.035	1.036	0.039
120	0.859	0.023	1.016	0.051	0.923	0.080	0.946	0.103	1.051	0.104	1.091	0.027	1.042	0.027

Table C-9. Summary of the physiological parameters for neonatal trichloroacetic acid 25 µg/ml experiments, expressed as a fraction of baseline values (N=6).

Time (min)	CF		HR		DP		MVO_2		dP/dT		PR Interval		QRS Duration	
	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE
0	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000
10	0.945	0.032	0.949	0.032	0.981	0.044	0.933	0.031	0.923	0.069	1.041	0.037	1.036	0.040
20	0.881	0.048	0.953	0.031	0.937	0.087	0.888	0.056	0.920	0.083	1.007	0.036	0.991	0.044
30	0.860	0.044	0.969	0.032	0.968	0.090	0.930	0.053	1.066	0.126	0.967	0.016	1.093	0.068
40	0.829	0.033	1.069	0.109	0.918	0.106	0.954	0.076	0.901	0.104	0.935	0.060	1.115	0.033
50	0.822	0.033	1.098	0.128	0.859	0.139	0.872	0.071	0.887	0.122	1.003	0.029	1.070	0.058
60	0.794	0.022	1.079	0.115	0.878	0.098	0.927	0.087	0.869	0.108	0.989	0.030	1.002	0.053
70	0.840	0.041	0.915	0.044	0.879	0.116	0.814	0.116	0.795	0.104	0.964	0.021	1.012	0.052
80	0.882	0.025	0.970	0.043	0.902	0.122	0.881	0.123	0.811	0.122	0.991	0.013	1.013	0.036
90	0.819	0.035	0.900	0.036	0.826	0.111	0.750	0.104	0.719	0.104	1.028	0.024	1.039	0.037
100	0.835	0.036	0.934	0.039	0.826	0.094	0.775	0.102	0.698	0.089	1.034	0.020	1.032	0.055
110	0.834	0.038	0.917	0.037	0.780	0.078	0.726	0.070	0.670	0.076	1.027	0.017	1.052	0.047
120	0.762	0.033	0.897	0.046	0.792	0.108	0.706	0.092	0.679	0.091	1.006	0.021	1.014	0.048

Table C-10. Summary of the physiological parameters for neonatal trichloroacetic acid 50 µg/ml experiments, expressed as a fraction of baseline values (N=6).

Time (min)	CF		HR		DP		MVO_2		dP/dT		PR Interval		QRS Duration	
	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE
0	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000
10	0.893	0.029	0.948	0.030	1.050	0.026	0.992	0.023	1.016	0.038	1.063	0.031	0.981	0.020
20	0.918	0.041	0.937	0.021	0.843	0.106	0.794	0.104	0.848	0.088	1.062	0.042	0.996	0.064
30	0.895	0.035	0.944	0.032	0.893	0.073	0.856	0.051	0.884	0.068	1.026	0.046	1.015	0.039
40	0.885	0.038	0.947	0.023	1.028	0.050	0.959	0.038	1.053	0.070	1.033	0.018	1.024	0.065
50	0.875	0.041	0.942	0.021	0.974	0.058	0.912	0.039	0.931	0.057	1.009	0.025	1.051	0.052
60	0.839	0.060	0.877	0.048	0.963	0.106	0.822	0.065	0.821	0.074	1.124	0.068	1.052	0.062
70	0.978	0.064	0.927	0.037	0.977	0.117	0.890	0.088	0.970	0.110	1.018	0.037	1.026	0.043
80	0.896	0.046	0.915	0.043	1.048	0.107	0.941	0.063	1.034	0.111	1.063	0.046	1.040	0.020
90	0.856	0.055	0.892	0.052	1.018	0.099	0.887	0.054	0.939	0.088	1.037	0.049	1.051	0.042
100	0.854	0.051	0.872	0.040	0.985	0.094	0.843	0.048	0.880	0.086	0.995	0.037	1.086	0.039
110	0.856	0.049	0.894	0.049	1.029	0.088	0.901	0.045	0.869	0.059	1.013	0.033	1.051	0.044
120	0.808	0.054	0.862	0.059	0.991	0.082	0.844	0.062	0.822	0.050	1.034	0.040	0.993	0.030

Table C-11. Summary of the physiological parameters for neonatal trichloroacetic acid 100 µg/ml experiments, expressed as a fraction of baseline values (N=7).

Time (min.)	CF		HR		DP		MVO_2		dP/dT		PR Interval		QRS Duration	
	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE
0	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000
10	0.921	0.026	0.954	0.014	0.969	0.029	0.952	0.041	0.936	0.023	0.996	0.027	1.040	0.036
20	0.839	0.023	0.956	0.013	0.938	0.029	0.935	0.036	0.962	0.029	1.032	0.026	1.084	0.047
30	0.838	0.037	0.957	0.024	0.854	0.030	0.889	0.042	1.012	0.071	1.033	0.030	1.035	0.049
40	0.838	0.036	0.962	0.024	0.832	0.031	0.879	0.041	1.032	0.110	1.027	0.054	1.004	0.033
50	0.821	0.047	0.957	0.017	0.807	0.035	0.865	0.043	1.005	0.118	1.155	0.104	1.036	0.036
60	0.830	0.043	0.946	0.011	0.782	0.037	0.845	0.040	0.928	0.105	1.186	0.094	1.043	0.050
70	0.905	0.044	0.986	0.030	0.735	0.036	0.840	0.040	0.911	0.106	1.123	0.093	0.978	0.034
80	0.847	0.051	0.967	0.022	0.743	0.031	0.836	0.033	0.796	0.048	1.044	0.053	0.952	0.019
90	0.833	0.054	0.933	0.045	0.701	0.048	0.798	0.033	0.775	0.045	1.091	0.075	0.990	0.034
100	0.824	0.051	0.935	0.031	0.683	0.045	0.790	0.034	0.747	0.061	1.066	0.050	0.966	0.035
110	0.809	0.052	0.906	0.037	0.672	0.050	0.770	0.033	0.716	0.042	1.072	0.077	0.976	0.032
120	0.749	0.055	0.861	0.019	0.653	0.045	0.738	0.036	0.668	0.042	1.066	0.086	1.059	0.019

Table C-12. Summary of the physiological parameters for neonatal trichloroacetic acid 150 µg/ml experiments, expressed as a fraction of baseline values (N=7).

Time (min.)	CF		HR		CP		MVO_2		dP/dT		PR Interval		QRS Duration	
	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE	Ave.	SE
0	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000
10	0.956	0.033	0.984	0.022	1.089	0.042	1.070	0.029	1.004	0.053	0.987	0.017	1.073	0.037
20	0.942	0.034	0.959	0.020	1.048	0.076	0.999	0.056	1.035	0.038	1.065	0.040	1.019	0.030
30	0.927	0.039	0.961	0.032	1.091	0.072	1.039	0.051	1.105	0.033	1.059	0.038	1.112	0.032
40	0.918	0.054	0.968	0.030	1.077	0.102	1.030	0.071	1.042	0.070	1.082	0.022	1.047	0.031
50	0.907	0.032	0.945	0.024	1.079	0.081	1.012	0.057	1.002	0.063	1.069	0.021	1.125	0.038
60	0.886	0.045	0.946	0.032	1.099	0.080	1.026	0.051	1.007	0.063	1.069	0.034	1.091	0.042
70	1.003	0.036	1.003	0.029	1.061	0.074	1.055	0.047	1.078	0.062	1.072	0.037	1.032	0.040
80	0.934	0.073	1.007	0.025	1.074	0.096	1.075	0.079	1.195	0.104	1.078	0.047	1.013	0.062
90	0.931	0.052	0.967	0.021	1.147	0.078	1.106	0.066	1.218	0.110	1.058	0.029	0.990	0.049
100	0.900	0.056	0.939	0.010	1.129	0.078	1.061	0.073	1.201	0.115	1.040	0.021	1.001	0.031
110	0.865	0.052	0.916	0.032	1.091	0.081	0.991	0.058	1.178	0.093	1.049	0.018	1.032	0.035
120	0.840	0.052	0.925	0.036	1.086	0.083	0.999	0.075	1.142	0.097	1.053	0.016	1.060	0.029

Table C-13. Maximal neonatal pharmacodynamic effects for control, chloral hydrate (50, 100 and 200 μg/ml), trichloroethanol (25, 50, 100 and 150 μg/ml) and trichloroacetic acid (25, 50, 100 and 150 μg/ml) experiments.[§]

Experiment	CF Ave. SE	HR Ave. SE	DP Ave. SE	MVO_2 Ave. SE	dP/dT Ave. SE	PR Ave. SE	QRS Ave. SE
NEOCONTROL	0.974 0.007	0.990 0.004	1.005 0.004	0.996 0.006	0.997 0.003	1.001 0.003	0.985 0.010
NEOCH 50 [μg/ml]	0.651 0.018	0.860 0.030	0.828 0.079	0.760 0.070	0.836 0.067	1.183 0.028	1.091 0.030
100	0.813 0.029	0.840 0.051	0.765 0.094	0.757 0.090	0.806 0.063	1.061 0.060	1.139 0.034
200	0.864 0.018	0.698 0.067	0.863 0.087	0.689 0.100	0.841 0.091	1.111 0.034	1.063 0.024
NEOTCE 25 [μg/ml]	0.826 0.022	0.855 0.045	0.925 0.078	0.830 0.062	0.876 0.068	1.113 0.029	1.054 0.044
50	0.882 0.024	0.813 0.041	0.880 0.101	0.735 0.110	0.811 0.084	1.188 0.058	1.067 0.030
100	0.860 0.013	0.801 0.081	0.782 0.138	0.688 0.125	0.613 0.111	1.147 0.069	1.108 0.031
150	0.830 0.044	0.847 0.029	0.806 0.047	0.736 0.045	0.871 0.038	1.106 0.012	1.111 0.045
NEOTCA 25 [μg/ml]	0.768 0.029	0.916 0.028	0.761 0.101	0.750 0.046	0.710 0.086	1.073 0.037	1.117 0.038
50	0.796 0.046	0.849 0.040	0.829 0.102	0.739 0.087	0.776 0.078	1.158 0.064	1.105 0.052
100	0.786 0.032	0.928 0.015	0.773 0.036	0.735 0.040	0.848 0.058	1.218 0.094	1.096 0.039
150	0.869 0.018	0.918 0.029	0.937 0.083	0.912 0.067	0.912 0.052	1.117 0.036	1.158 0.030

[§] Maximal effects were determined in the treatment phase of the protocol only, and are expressed fractions of the respective baseline values.

APPENDIX D

- REPRESENTATIVE CHROMATOGRAMS
- ASSAY VALIDATION
- GC STANDARD CURVES

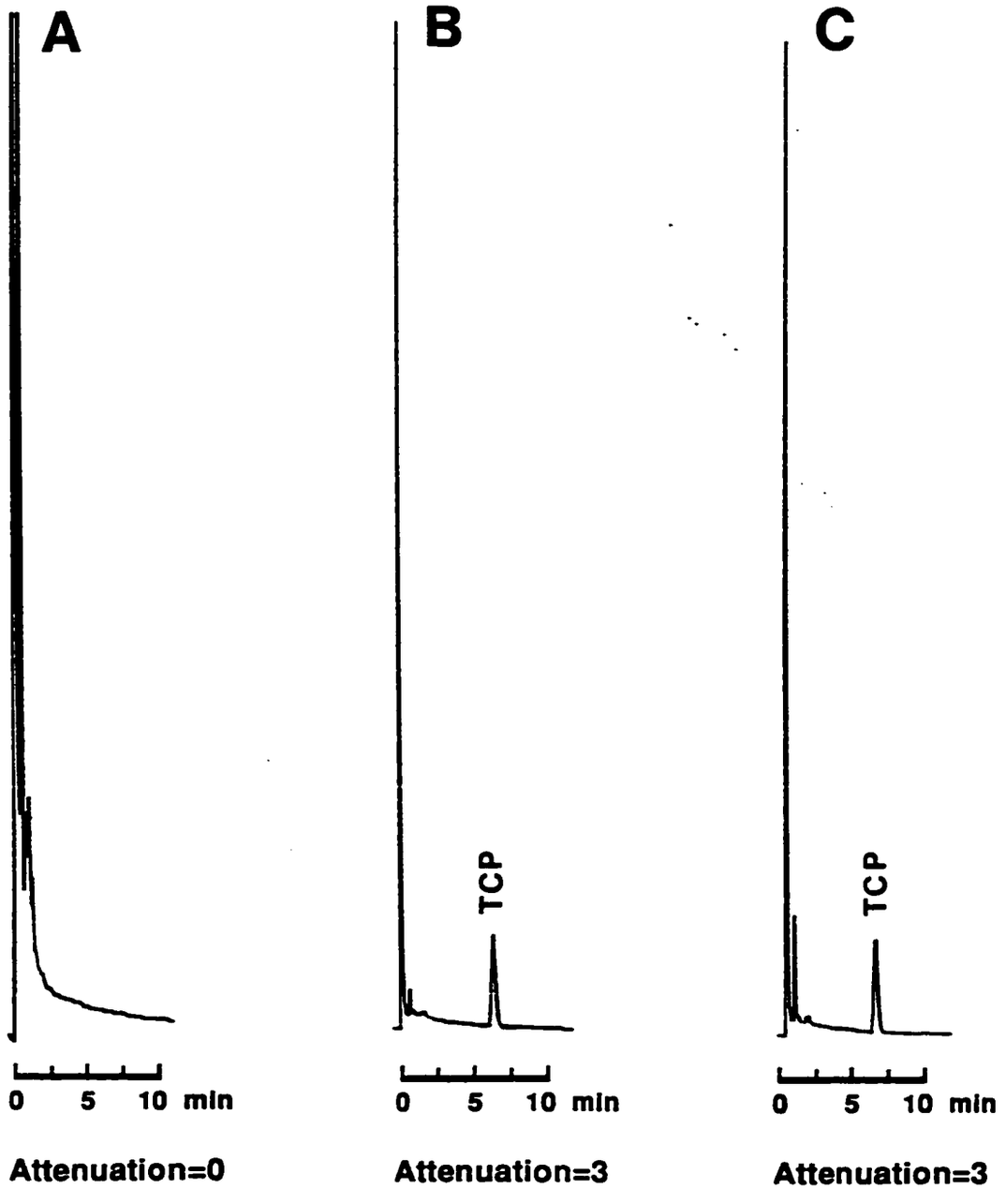


Figure D-1. Model chromatograms for (A) 2 µl freshly distilled diethyl ether; (B) 1 µl extracted blank buffer and 1,2,3-trichloropropane (TCP - internal standard); (C) 1µl derivatized and extracted blank buffer with TCP.

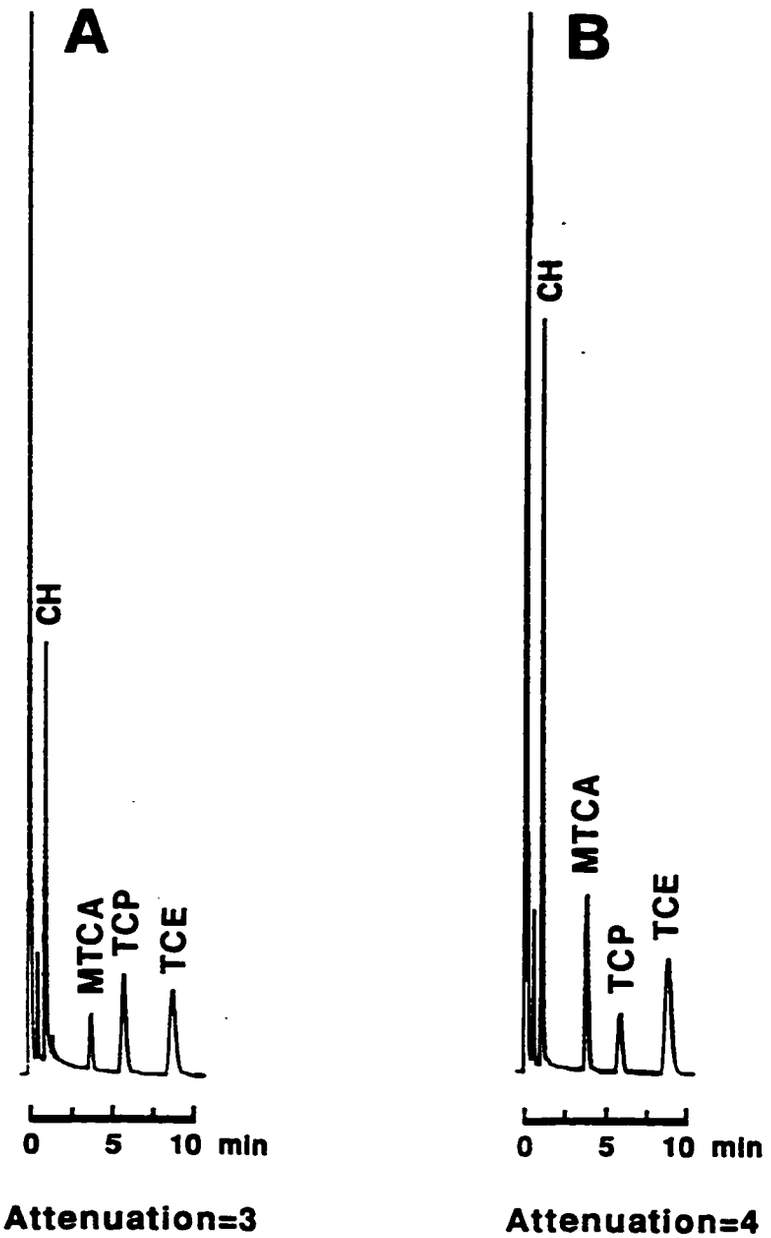


Figure D-2. Model chromatograms for derivatized extracts (from buffer) of (A) 10 µg/ml standards; (B) 25 µg/ml standards (CH=chloral hydrate; MTCA=methyl trichloroacetate; TCP=trichloropropane; TCE=trichloroethanol).

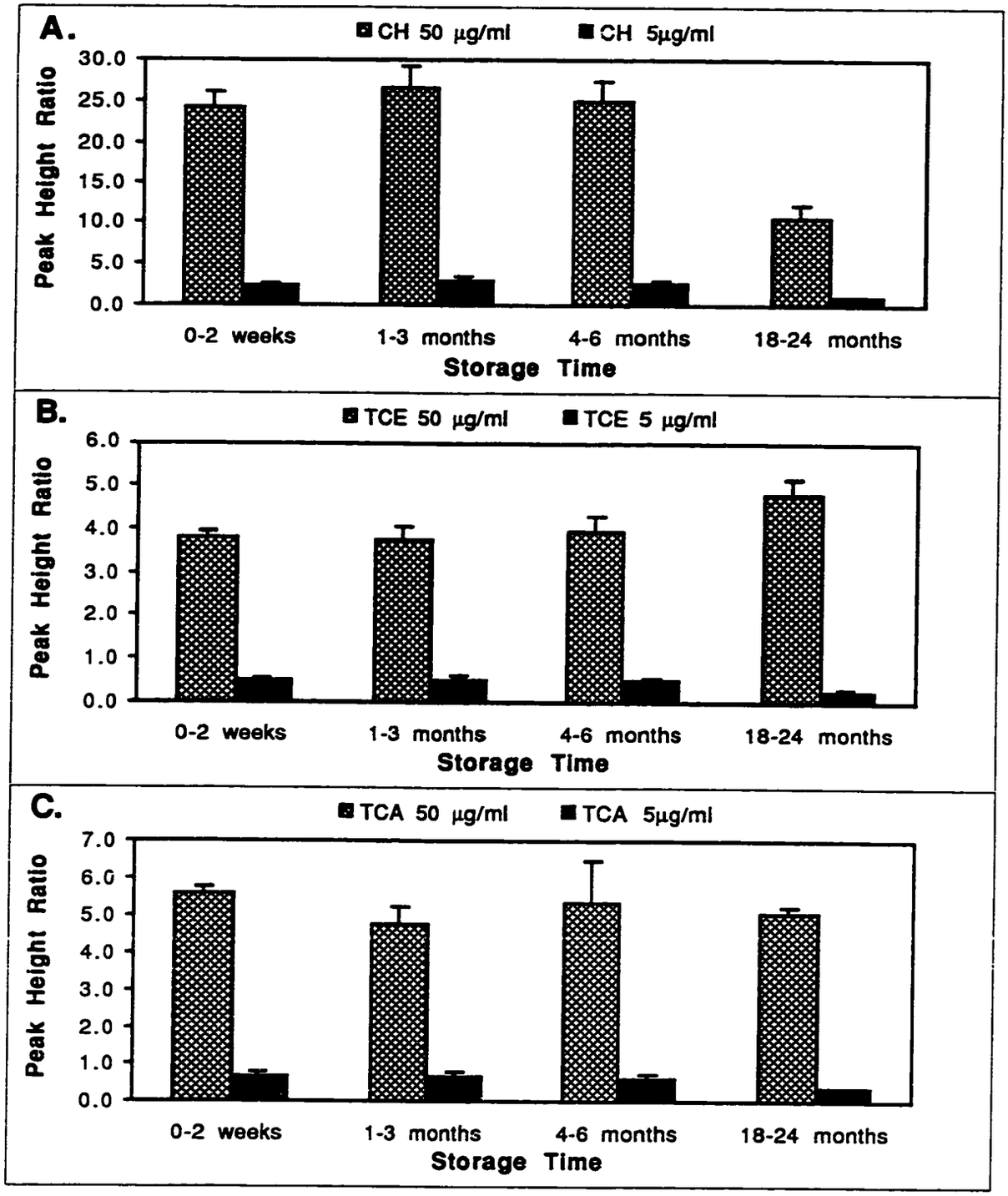


Figure D-3. Intraday variability and stability data for 5 and 50 µg/ml chloral hydrate (A), trichloroethanol (B) and trichloroacetic acid (C) standards. Samples were analyzed following storage at -70° C for periods ranging from 0 days (freshly prepared standards) to 24 months. Error bars represent the standard error of the group means. N=5-8 for each group.

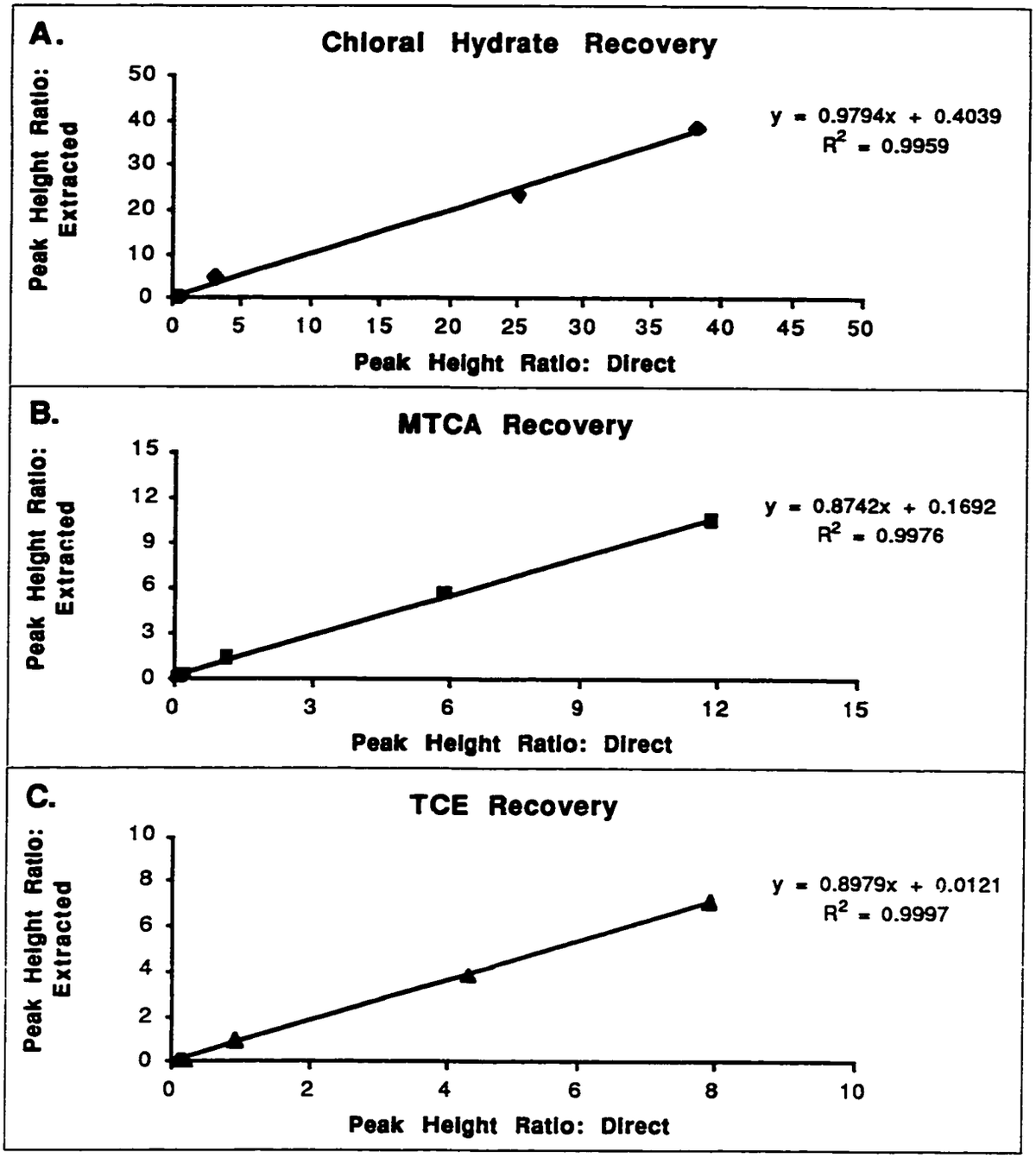


Figure D-4. Recovery data for 0.5, 1.0, 10.0, 50.0 and 100.0 µg/ml chloral hydrate (A), methyl trichloroacetate (B) and trichloroethanol (C) standards. Standards were either extracted and analyzed gas chromatographically as described, or equivalent amounts of each analyte (with internal standard) were prepared in absolute ethanol and directly injected onto the gas chromatograph. The slope of the linear regression line reflects the proportionate error of the analytical method, while the y-intercept is indicative of method bias (slope=1 and y-intercept=0 for a method with 100% recovery). N=5-10 per group.

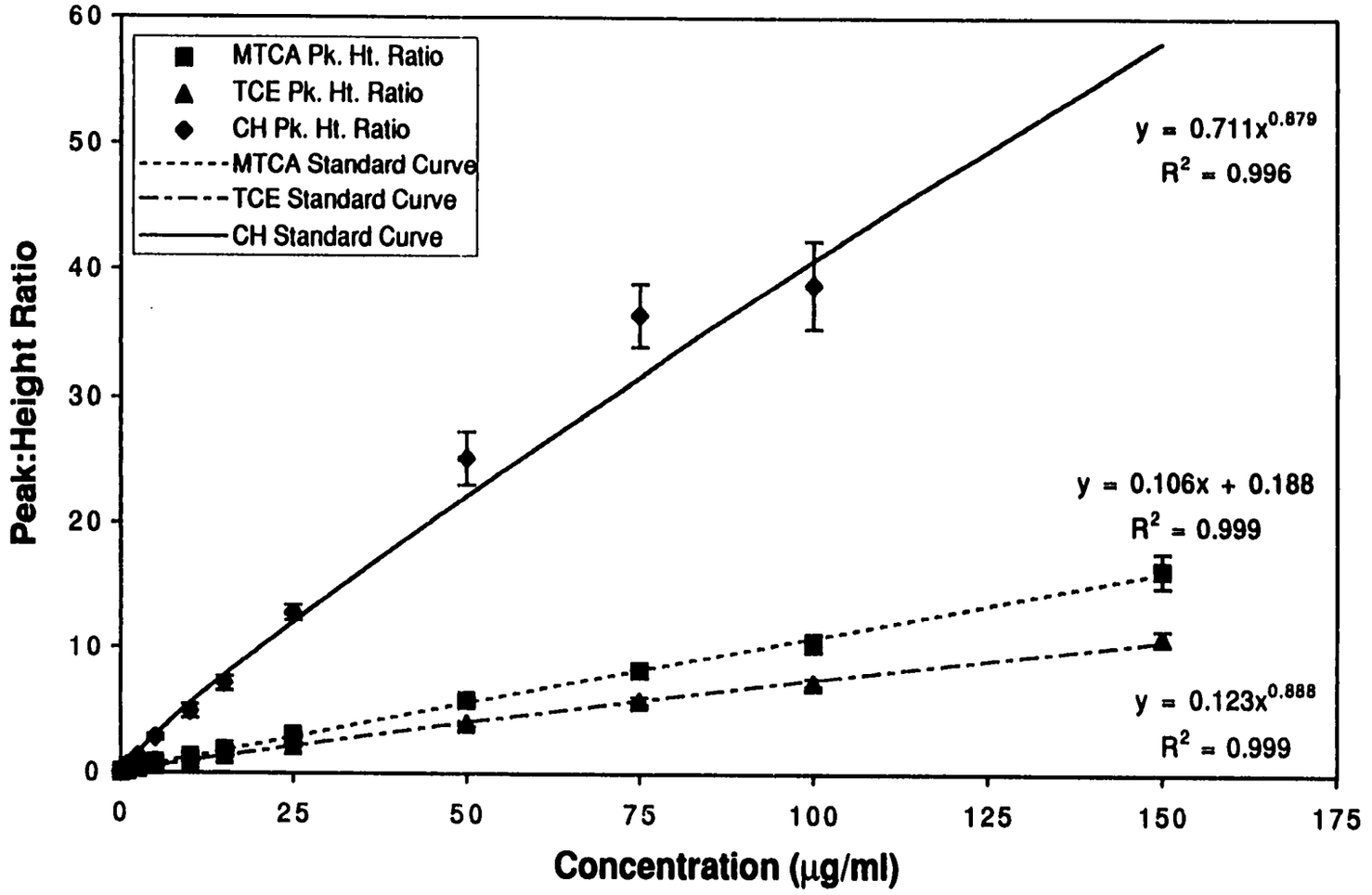


Figure D-5. Electron-capture gas chromatographic standard curves for CH, TCE and MTCA (0 day-6 months storage). The equations and coefficients of determination (R^2) accompany the regression curves.

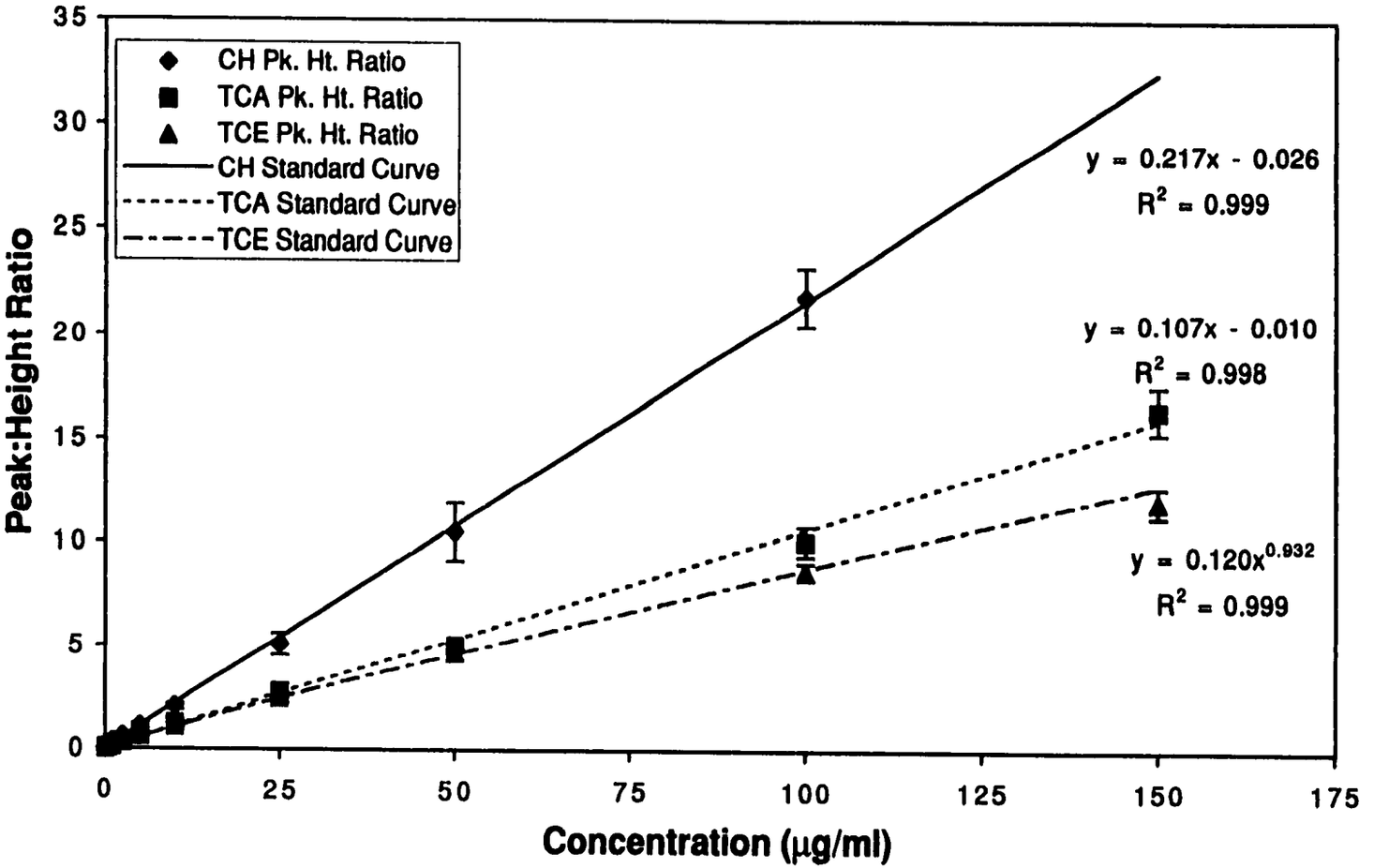


Figure D-6. Electron-capture gas chromatographic standard curves for CH, TCE and MTCA (>12 months storage). The equations and coefficients of determination (R^2) accompany the regression curves.