Evaluation of Cardiac Sensitisation Test Methods

Technical Report No. 105

ISSN-0773-8072-105
Brussels, September 2009

ECETOC TECHNICAL REPORT NO. 105

European Centre for Ecotoxicology and Toxicology of Chemicals

4 Avenue E. Van Nieuwenhuyse (Bte 6), B-1160 Brussels, Belgium.

Evaluation of cardiac sensitisation test methods

CONTENTS

EXECUTIVE SUMMARY **1**

1. SUMMARY **2**

2. INTRODUCTION **4**

2.1 Historical perspective 4
2.1.1 Definition of cardiac sensitisation *4*
2.1.2 Animal studies *4*
2.1.3 Clinical experience and epidemiology studies *5*

3. KEY PROTOCOL REQUIREMENTS **7**

3.1 Preparation of animals 7
3.2 Preparation of test material 7
3.3 Recording the electrocardiogram 7
3.4 Administration of adrenaline 8
3.5 Limitations of protocol 8

4. CURRENT PROTOCOLS AND PROS AND CONS **10**

4.1 Current protocols – Example I 10
4.2 Current protocols – Example II 11
4.3 Current protocols – Example III 13

5. DISCUSSION OF KEY FACTORS **14**

5.1 Species 14
5.2 Number of animals 15
5.3 Duration of exposure 16
5.4 Test material concentration 18
5.5 Selection of the dose of adrenaline 18
5.6 Response criteria – What constitutes a positive response? 20
5.6.1 Criteria used in dog studies *20*
5.6.2 Comparison of dog criteria to human definition *21*

6. REPRODUCIBILITY OF FINDINGS **22**

6.1 1,1-Dichloro-1-fluoroethane (HCFC-141b) 22
6.2 1,1,1,2-Tetrafluoroethane (HFC-134a) 23

7. PHARMACOKINETICS **24**

7.1 Physiologically-based biokinetic modelling and risk assessment considerations 24
7.1.1 Introduction *24*
7.1.2 Model construction and data requirement *25*
7.1.3 Modelling of cardiac sensitisation and risk assessment in humans *28*
7.1.4 Concluding comment on PBBK model approach and its use in risk assessment *30*

8. RISK ASSESSMENT **31**

8.1 Interpretation of data 31
8.2 Human considerations 32
8.3 Other factors 33
8.4 Summary 33

9. MECHANISM OF ACTION **34**

9.1 Introduction 34
9.2 Cardiac sensitisation to catecholamines by volatile anaesthetics *in vivo* 34
9.3 Mechanisms of arrhythmogenesis in general 35
9.4 Interaction of volatile anaesthetics with cardiac targets 35
9.4.1 Adrenergic and muscarinic receptor-mediated signal transduction *35*
9.4.2 Catecholamine reuptake *36*
9.4.3 Cardiac sodium and calcium channels *36*
9.4.4 Cardiac repolarising K^+ currents: I_{Kr}, I_{Ks}, inward rectifiers, I_{to}, and two-pore-domain channels *37*
9.4.5 Action potentials, conduction, and intercellular coupling *38*
9.5 Mechanisms contributing to cardiac sensitisation by volatile anaesthetics 39

10. ALTERNATIVE APPROACHES **41**

10.1 Political and regulatory environment 41
10.2 Biochemical *in vitro* assays and *in silico* structure-activity prediction 41
10.3 Cell- and tissue-culture-based *in vitro* and *ex vivo* models 42
10.4 *Ex vivo* pro-arrhythmia models 43
10.4.1 Isolated perfused heart (Langendorff heart model) *43*
10.4.2 Purkinje fibres *44*
10.5 *In vivo* models 45

11. CONCLUSIONS AND RECOMMENDATIONS **46**

11.1 The adrenaline-treated dog is the current test system of choice 46
11.2 The test is highly conservative (protective) 46
11.3 Interpretation of results should be done by experienced practitioners 47
11.4 Cardiac Sensitisation test results can be further interpreted using kinetic models 47
11.5 Alternatives to current test system 47

12. BIBLIOGRAPHY **48**

12.1 References quoted 48
12.2 References not quoted 68

LIST OF SPECIAL ABBREVIATIONS 69

APPENDIX A: CONVERSION FACTORS FOR VAPOUR CONCENTRATIONS IN AIR 71

APPENDIX B: SOME HALOCARBONS TESTED FOR CARDIAC SENSITISATION 72

APPENDIX C: NAMING AND NUMBERING SYSTEM FOR FLUOROCARBON COMPOUNDS 74

APPENDIX D: CARDIAC SENSITISATION SCORING 77

APPENDIX E: ARRHYTHMOGENIC DOSES OF (NOR)ADRENALINE IN HUMANS AND DOGS 82

APPENDIX F: POTENCY OF VOLATILE ANAESTHETICS AT CARDIAC ION CHANNELS / TARGETS 84

MEMBERS OF THE TASK FORCE 90

MEMBERS OF THE SCIENTIFIC COMMITTEE 91

EXECUTIVE SUMMARY

Cardiac sensitisation means that the normal heart rhythm becomes perturbed, for example, when high concentrations of some halogenated or unsubstituted hydrocarbon vapours are inhaled, for a short time, in combination with situations causing high internal adrenaline levels (stress). The resulting cardiac arrhythmias may be fatal. Therefore, the cardiac sensitisation potential (and other toxicological properties) of development fluorocarbon products needs to be known before those products can be safely used, especially in air-conditioning and fire-fighting applications.

This report reviews how cardiac sensitisation studies have been conducted and the way in which those test results are used in the prediction of human risk. Critical aspects of the test protocol are the selection of the dose of adrenaline used to 'challenge' the heart and the definition of a positive response (i.e. cardiac arrhythmia). Risk assessment of cardiac sensitisation is based on the extrapolation of the animal data to humans, but should also take account of individual susceptibility and health status, and other factors such as medication. The current protocols appear to provide accurate, robust scientific information and are designed conservatively with built-in safety factors. Thus no additional safety factors need to be applied in the risk assessment process. For improved risk assessment, human blood levels of the inhaled compounds can be estimated using biokinetic modelling techniques. This kind of modelling can also account for sensitive sub-populations.

Little is known about the biological mechanism of the cardiac sensitising effects of halogenated and unsubstituted hydrocarbons. The report reviews several aspects and possible contributing factors in detail. Compared to volatile anaesthetics, which have an adverse impact on cardiac electrophysiology, cardiac sensitisation seems to be a complex event that is not fully understood at present.

There is no clear alternative test system for cardiac sensitisation. The report discusses the utility of various possible alternatives to replace or refine the current adrenaline-treated dog model.

1. SUMMARY

The term ‘cardiac sensitisation’ is used to refer to the phenomenon whereby exposure to high concentrations of many halogenated and unsubstituted hydrocarbons may cause the heart to be transiently more sensitive than normal to the arrhythmogenic effects of endogenous catecholamines. This ‘sensitisation’ coupled with elevated levels of circulating adrenaline can result in the development of serious and, sometimes, fatal cardiac arrhythmias for a short period following exposure to sufficiently high concentrations of these compounds.

Although it is clearly a non-standard toxicological end-point, cardiac sensitisation potential is considered to be a critical end-point to be defined during the development of fluorocarbon products, especially those for use in air-conditioning and fire-fighting applications. Consequently, experimental protocols have been developed to assess the cardiac sensitisation potential of development products, using the dog as the animal model.

The goals of this report are:

- To conduct a critical review of the existing protocols for studies in the dog using acknowledged experts in the field.
- To recommend the most appropriate protocol for the conduct of the dog study.
- To provide guidance on interpretation of the data from the study and its use in risk assessment processes.
- To comment on the validity of the physiologically-based biokinetic (PBBK) [a] model that has been developed for use with the study.
- To discuss the likely mechanism of the effect with a view to recommending an approach to the development of an alternative method for the assessment of the effect.

The phenomenon was first identified as a consequence of the anaesthetic use of chloroform. This report reviews the early experimental studies that characterised the phenomenon and the reports of its occurrence in humans. The protocols that are currently in use have been critically appraised and the key protocol requirements discussed in detail. It is concluded that, currently, there is no clear alternative animal model to the adrenaline-treated dog. One critical aspect of the protocol is the selection of the dose of adrenaline used to ‘challenge’ the heart. It is concluded that it is most appropriate to titrate the challenge dose of adrenaline used for each dog in order to reduce individual variability in response or to challenge each dog with a range of adrenaline concentrations. A second critical aspect is the definition of a positive response (i.e. cardiac arrhythmia). Other aspects of the protocol that are discussed are group size, concentration and duration of exposure to the test substance and the limitations of the protocol.

[a] See list of special abbreviations used in this report

The use of a PBBK model to predict human blood levels of the substance in question is described. This approach facilitates the extrapolation of data derived using the dog model to humans and allows the response of potentially sensitive sub-populations to be taken into account in the risk assessment process. Data to build the PBBK model can be derived by measuring arterial blood levels of the test material at relevant test concentrations in the dog studies and transposing them to humans using relevant biokinetic constants that are either known or can be measured.

An important consideration is the way in which the results of these studies are used in the prediction of human risk. The risk assessment process regarding cardiac sensitisation should take into account the extrapolation of the animal data to humans, individual susceptibility, the health status of the individual and other factors such as medication. It is argued that the normal practice of applying a safety factor to an observed no-effect concentration is not the most appropriate way to use the data from the protocols based on the dog model in the prediction of human risk. The current protocols appear to provide accurate, robust scientific information relative to the cardiac sensitisation potential of chemicals of interest and, with the use of the PBBK model, are valid predictors of cardiac sensitisation potential for use in human risk assessment. The protocols are designed conservatively with built-in safety factors and thus no additional safety factors need to be applied in the risk assessment process. It is acknowledged that the current protocols might be further developed to improve their applicability and utility as risk assessment tools.

Little is known about the underlying mechanisms of the cardiac sensitising effects of halogenated and unsubstituted hydrocarbons. The report reviews a number of potential targets of effects including receptor-mediated signal transduction, neurotransmitter re-uptake, impact on sodium and calcium channels in cardiac muscle, cardiac muscle potassium repolarisation currents and the potential impact on cardiac action potentials, conduction velocity and intercellular coupling. The evidence from volatile anaesthetics suggests an adverse impact on cardiac electrophysiology that may have relevance to the other halocarbons. It is concluded that cardiac sensitisation is a complex event involving multiple possible mechanisms and that the exact mechanism targeted by halocarbons is not fully understood at present.

The report recognises that current ethical and political considerations favour the development of alternative testing strategies based on the concept of the ‘3Rs’ – the replacement, refinement and reduction of the use of animals in research. The current utility of *in silico*, *in vitro*, cell or tissue culture, *ex vivo* and alternative *in vivo* models as alternatives to the dog model are considered. It is concluded that it may be possible to develop one or more alternatives to the current protocol in the future. However, it is recognised that any viable alternative will require full validation against the historical cardiac sensitisation data and will require acceptance by the scientific and regulatory communities before it can be utilised in risk assessment.

2. INTRODUCTION

2.1 Historical perspective

The phenomenon now referred to as cardiac sensitisation was first described by Levy and Lewis (1911-1912) and Levy (1913) when they demonstrated in cats that adrenaline and chloroform (≈ 0.5 - 1.0% [5,000 - 10,000 ppm; 24,400 - 48,800 mg/m^3] [a]), when administered separately, did not produce serious heart arrhythmias but, when administered together, could produce fatal ventricular fibrillation. They found that the arrhythmias sometimes advanced to ventricular fibrillation when a dose of 65 µg of adrenaline was given intravenously during the administration of chloroform.

Based on these studies, Levy and Lewis were of the opinion that many of the sudden deaths that occurred during chloroform anaesthesia were caused by chloroform increasing the irritability of the heart muscle, making it more sensitive to adrenaline, thus resulting in the development of fatal arrhythmias, especially during light anaesthesia.

2.1.1 Definition of cardiac sensitisation

The term 'cardiac sensitisation' refers to the possibility of an enhanced risk of arrhythmogenic effects usually resulting from an exposure to a volatile substance or a drug. In particular, it is used to refer to the phenomenon whereby exposure to high concentrations of many halogenated and unsubstituted hydrocarbons may cause the heart to be transiently more sensitive than normal to the arrhythmogenic effects of endogenous catecholamines. These circumstances can result in the development of serious and sometimes fatal cardiac arrhythmias upon exposure to sufficiently high concentrations of these compounds coupled with elevated levels of adrenaline. The exact mechanism involved in producing these cardiac arrhythmias is not known, but several possibilities have been suggested and are discussed in Section 9.

2.1.2 Animal studies

Many years after the work of Levy and Lewis, other investigators (Meek *et al*, 1937; Waters *et al*, 1943; Morris *et al*, 1953; Raventos, 1956; Bamforth, 1961) found that newer anaesthetic agents such as cyclopropane, trichloroethylene, halothane and methoxyflurane could produce the same kind of cardiac effect in humans and animals as had been demonstrated with chloroform. Meek *et al* (1937) showed that diethyl ether, at most, only weakly sensitised the dog heart to adrenaline. At about this same time, other investigators (Chenoweth, 1946; Garb and Chenoweth, 1948;

[a] Converted following Appendix A (1% = 10,000 ppm, by volume)

Krantz *et al*, 1948; Carr *et al*, 1949; Burgison *et al*, 1955) discovered that many common industrial halocarbons and unsubstituted hydrocarbons such as gasoline, carbon tetrachloride and benzene also produced cardiac sensitisation in experimental animals.

Most of these later studies utilised the dog rather than the cat as the animal model. Typically, the animals were exposed to the test compound by inhalation and adrenaline was administered intravenously, while an electrocardiogram (ECG) was obtained to monitor the heart rhythm during the procedure. The dose of adrenaline and the exposure duration varied amongst the different studies and are discussed in Sections 5.3 and 5.5.

Since then, numerous halocarbons have undergone cardiac sensitisation testing (Appendix B).

It is recognised that the assessment of possible cardiotoxic potential of new drugs is a routine test for the pharmaceutical industry. ICH guideline S7B (2005) describes a number of screening studies that are used for the evaluation of anti- or pro-arrhythmic effects during the early phase of the pre-clinical development of drugs. The guideline also recommends that *in vivo* data from relevant animal models, e.g. the dog, are the preferred basis for the risk assessment of cardiotoxic potential of human pharmaceuticals. To date, these guideline studies have not found utility in the assessment of cardiac sensitisation potential of volatile industrial chemicals. Their future utility in this respect is assessed in Section 10.

2.1.3 Clinical experience and epidemiology studies

During the past century, numerous instances of sudden death have been reported in industrial settings where workers were accidentally exposed to high levels of volatile unsubstituted and halogenated hydrocarbons such as benzene, carbon tetrachloride and trichloroethylene (Hamilton, 1925; National Safety Council, 1926; McCord, 1932; Feil, 1933; Hermann and Vial, 1934; Hermann and Vial, 1935; Bell, 1951; Hoschek, 1962; Kleinfeld and Tabershaw, 1954; Schollmeyer, 1960). In most cases, the cause of these deaths was not determined with certainty and was often considered to be a lack of oxygen and/or central nervous system depression.

In the late 1960s and early 1970s, numerous sudden deaths were reported in teenagers who inhaled ("sniffed") various products to achieve a chemically induced euphoria. Bass (1970) reported 110 cases of sudden death, mostly in teenagers. The chemicals involved included toluene, benzene, gasoline, 1,1,1-trichloroethane and chlorofluorocarbon aerosol propellants such as dichlorodifluoromethane (CFC-12) and trichlorofluoromethane (CFC-11)[a]. The solvent trichlorotrifluoroethane (CFC-113) was also reported to cause sudden deaths in situations where

[a] The naming and numbering convention is explained in Appendix C

workers were accidentally exposed to very high concentrations (NIOSH, 1989; Kaufman *et al*, 1994).

In most of the cases of sudden death, no cause could be determined upon pathological examination. Several causes were postulated including laryngeal oedema or spasm caused by irritation from the inhaled aerosol droplets, anoxia, central nervous system depression and fatal heart arrhythmias. The deaths were somewhat surprising since the chlorofluorocarbons in particular were known to be compounds of low toxicity through previously conducted laboratory animal studies and many years of safe use as refrigerants, solvents, foam blowing agents and aerosol propellants.

The lack of autopsy findings plus the circumstances surrounding the deaths (high levels of exposure probably coupled with excitement or stress) led researchers to conduct laboratory studies of various halocarbons and hydrocarbons to investigate the cause(s) of the deaths.

Subsequently, several clinical studies of refrigeration repairmen and aerospace workers have not shown any significant effects on the heart that were considered attributable to exposure to fluorocarbon refrigerants and other fluorocarbon compounds (Antti-Poika *et al*, 1990; Edling *et al*, 1990; Egeland *et al,* 1992).

3. KEY PROTOCOL REQUIREMENTS

The principles involved in most of the protocols currently used for evaluation of cardiac sensitisation of volatile unsubstituted and halogenated hydrocarbons are similar. They involve inhalation exposure of dogs to the test material, intravenous administration of adrenaline and recording of an electrocardiogram.

3.1 Preparation of animals

In order for dogs to be exposed to the test gas or vapour using a face-mask, they must first be trained to accept the experimental procedure. The dogs are normally restrained in a canvas sling during the test procedure to ensure that ECG leads can be attached and, if necessary, adjusted. Thorough training of the dogs is essential prior to performing cardiac sensitisation studies and this usually requires gradual accommodation (2 - 4 wk) of the animals to the restraint procedures and the face-mask. This approach helps to ensure that endogenous adrenaline levels are as stable as possible throughput the procedure. Recent research has shown that reliable monitoring of the ECG by telemetry is possible in the dog during inhalation exposure (Meecham *et al*, 2006) and that less stressful methods of restraint are feasible (Hussain *et al*, 2006). The use of such procedures may result in lower variability in response, as endogenous adrenaline levels would be less variable due to the minimisation of stress.

3.2 Preparation of test material

Preparation of the test gas or vapour mixture can be undertaken using a dynamic dilution system with real-time monitoring of the concentration by infra-red gas absorption; but, this may be an unstable method and may be difficult to control, particularly with less volatile materials. An alternative approach is to prepare sufficient test gas dilution for the exposure in a large Tedlar gas-bag and have the dog breathe this mixture via a one-way valve connected to the face-mask. This method has the advantage that the gas mixture can be prepared and analysed in advance of performing the test. However, the face-mask must have an airtight seal to the dog's muzzle; otherwise, room air may be drawn into the mask during respiration thus diluting the test atmosphere.

3.3 Recording the electrocardiogram

Another key requirement is the consistent recording of the electrocardiogram throughout the test procedure. This requirement is normally accomplished using conventional ECG leads (Lead II)

affixed to the dog with blunt spring clips, although any method that assures robust connection and electrical contact is acceptable. As mentioned above, the use of telemetry may also be considered.

3.4 Administration of adrenaline

The way in which the dose of adrenaline is administered must be carefully controlled. As is discussed in Section 5.5, not only is the total dose of adrenaline administered important, but also the rate at which it is administered. The cardiac response depends on the concentration of adrenaline in the blood as it enters the heart chambers and capillaries serving the cardiac musculature. Therefore, consistent administration of the intravenous dose of adrenaline is very important for any series of studies designed to compare the cardiac response of different test materials. Ideally, the vein should be temporarily cannulated in order to ensure that the adrenaline solution can be delivered at precisely the correct time and without infiltration into the surrounding tissue. The rate of delivery should be controlled either by a skilled operator injecting the dose manually while using a stopwatch or by the use of a syringe drive to inject the solution at a constant rate.

3.5 Limitations of protocol

One should recognise that however sophisticated the details of the protocol for this type of study, certain inherent limitations within the study design restrict the accuracy of the outputs. For example, the end-point for any particular test session is the observation of a 'positive response'. However, the definition of a positive response in this type of study is not straightforward. The positive response is a particular type of waveform or rhythm seen in an electrocardiogram and is therefore, to some extent, subjective and dependent on the experience of the experimenter. What constitutes a positive response is discussed in Section 5.6. Secondly, the order in which particular gas concentrations are tested and the actual gas concentrations that are used can influence the final conclusion of the study. For example, if a test material has an approximate lowest effect level and no effect level of 4% and 2% (40,000 ppm and 20,000 ppm, respectively) but only gas concentrations of 1% and 5% (10,000, 50,000 ppm) are tested, a positive response would be observed at 5% with no response at 1%. The conclusion of the study would be that the lowest observed effect level (LOEL) and no observed effect level (NOEL) would be 5% and 1% respectively. This outcome is the consequence of the fact that the result of this type of test is quantal in that a positive response in 1 animal is a positive result for the study. Thus, one cannot interpolate theoretically between the experimental NOEL and LOEL to define either more accurately. These concepts are discussed in more detail in later sections, but this brief introduction serves as a reminder that the results of cardiac sensitisation studies using this

protocol must be considered carefully and the details of the experimental procedures taken fully into account when attempting to interpret the results of the tests and use them for risk assessment.

4. CURRENT PROTOCOLS AND PROS AND CONS

4.1 Current protocols – Example I

One of the earlier standard cardiac sensitisation screening protocols is described in a publication by Reinhardt *et al* (1971). Beagle dogs that had been trained to accept a flow-through mask and stand in a sling were exposed to the test compound for a brief period of time and given an injection of adrenaline intravenously during the exposure. The actual sequence of events was as follows (Table 4.1).

Table 4.1: Cardiac sensitisation screening protocol (Reinhardt *et al*, 1971)

Time (min)	Action
0	Start ECG recording
2	Administer adrenaline (8µg/kgbw in 1 ml of normal saline in 9 s into a cephalic vein)
7	Start inhalation of test atmosphere
12	Administer adrenaline (8µg/kgbw in 1 ml of normal saline in 9 s into a cephalic vein)
17	Stop test atmosphere inhalation (exposure may be stopped earlier if a positive response is seen or if the dog is in distress). Stop ECG recording

The ECG findings after the challenge injection of adrenaline were compared with those that occurred following the control injection. Positive cardiac sensitisation responses were recorded when either a run of consecutive multiple ventricular beats, ventricular tachycardia, or ventricular fibrillation occurred following the challenge injection of adrenaline since these serious arrhythmias never occurred following a control injection. Additionally, a group of 13 dogs that breathed only room air throughout the 17-minute experimental routine and given the same 2 injections of adrenaline showed no serious arrhythmias. The dose of adrenaline used in these studies, 8 µg/kgbw, was similar to that used by other investigators in the past; for example 10 µg/kgbw given intravenously in a few seconds to about 1 minute (Meek *et al*, 1937; Raventos, 1956; Bamforth *et al*, 1961; Chenoweth, 1946; Krantz *et al*, 1948; Hermann and Vial, 1935; Phillips *et al*, 1946).

These studies were designed to screen compounds for their ability to produce cardiac sensitisation and to categorise them as to their relative potency as being weak, mild or strong cardiac sensitisers. Two or 3 exposure concentrations were used to provide a dose-response relationship so that the data would be more useful for risk assessment purposes.

Examples of the potency categorisation based on the results of a number of compounds tested are: CFC-11, strong; CFC-12, mild; and chloropentafluoroethane (CFC-115), weak. The threshold for cardiac sensitisation for these compounds was 5,000, 50,000 and 150,000 ppm (28,100, 247,000, 947,000 mg/m^3), respectively (Reinhardt *et al*, 1971).

In this study and in a subsequent study (Reinhardt *et al*, 1973), nearly all the compounds tested produced cardiac sensitisation at some concentration, most of them at high concentrations. All effect levels were seen at levels above those typically recommended for safe workplace exposures. In many cases, the effect levels were more than one order of magnitude higher than the concentrations recommended for worker exposure. In some cases, however, the ratio was smaller and yet these compounds were safely used for decades (e.g. FC-11). For each test substance, a dose-response effect was observed and a no-observed-effect-level was observed in most cases or could be estimated.

Additional studies were conducted with several common industrial solvents such as trichloroethylene, 1,1,1-trichloroethane, and CFC-113 using the same protocol as described above and were also found to produce cardiac sensitisation (Reinhardt *et al*, 1973). Two of the solvents tested, tetrachloroethylene and dichloromethane, did not produce cardiac sensitisation at 10,000 and 20,000 ppm (67,800, 79,200 mg/m^3), respectively. These concentrations caused the dogs to struggle and, thus, higher concentrations were not used.

4.2 Current protocols – Example II

When the test procedure was formalised and published in the early 1970s (Reinhardt *et al*, 1971), the recommended challenge dose of adrenaline was 8 µg/kgbw contained in 1 ml of normal saline administered at a constant rate over 9 seconds. In the early 1990s there was a major revival of interest in this test as the search for alternatives to the ozone-damaging CFCs gained momentum. Later, when the test was being redeveloped and validated (Hardy *et al*, 1994), a marked difference was noted in the response of individual dogs to adrenaline in the absence of test gas exposure. As a result, some of the early studies were carried out using adrenaline doses of 5 or 10 µg/kgbw delivered at a rate of 0.5 or 1 µg/s respectively, for more and less sensitive animals. Subsequently, further studies showed that inter-animal variation in the response to intravenous adrenaline alone was common. A review of the earlier literature supported this finding in that it was reported by Mullin *et al* (1972) that all the marked responses seen in the exercise experiments were seen in the same dog and that this dog had been noted to develop arrhythmias more readily than other dogs when used in the series of experiments described by Reinhardt *et al* (1971). Therefore a decision was made to develop a standard approach for the assessment of each dog's responsiveness to adrenaline and the selection of an appropriate dose for that dog. A cohort of 9 male dogs was assigned to each study. Depending on the number of studies being

carried out at the time, these animals could be selected from a colony retained for use on such studies or could be a delivery of 9 naive animals. As there were no lasting effects, the dogs were often re-used for a total of up to 5 studies. Each dog was trained to accept the restraint procedure and application of a face-mask. The dog was then fitted with ECG leads (Lead II). Solutions of adrenaline (0.1 ml/kgbw of an appropriate dilution administered at approximately 0.1 ml/s into a cephalic vein) were injected and the ECG recorded. The initial dose of adrenaline was 4 µg/kgbw. If the response to the first adrenaline challenge was too great, as defined by an excessive number of ectopic beats (more than approximately 10) or if, following 2 injections, there were no ectopic beats, the experimental session did not proceed further and the dog was allowed to rest for at least 20 minutes. The procedure was then repeated with an adrenaline dose half or double that previously given, except that the highest dose was 12 µg/kgbw (i.e. if there was no response at 8 µg/kg, this was followed by 12 µg/kgbw). Doses in excess of 12 µg/kgbw were found to cause marked tachycardia and very large QRS complexes followed by bradycardia. When a suitable dose of adrenaline had been selected for each dog, that dose was used for all subsequent work. Following determination of the appropriate adrenaline dose for the 9 dogs in the cohort, 6 were selected for use on the study. Whenever possible, the 6 selected dogs included dogs that were very responsive to adrenaline and also dogs that were less responsive. Some dogs were found to be very responsive to adrenaline and consistently produced several ectopic beats with adrenaline doses as low as 2 µg/kgbw. However, some dogs did not demonstrate an arrythmogenic effect from adrenaline administration alone at any dosage up to 12 µg/kgbw but displayed marked reflex bradycardia which limited the adrenaline dose that could be used. When the same dogs were used for a series of studies, the response of each individual dog to adrenaline alone was noted to be remarkably consistent and, thus, an individual dog's adrenaline dose even over the course of 5 separate studies rarely needed to be altered. Testing was undertaken using the appropriate adrenaline dose following the scheme described above in Section 4.1. Test atmospheres were premixed in a large (100 or 200 l) Tedlar gas-bag by mixing a measured quantity of the test substance with a measured quantity of air. The concentration of test substance was confirmed before and after the dosing session by chemical analysis. The test atmosphere was delivered to the test animal's mask via a one-way valve system. The initial concentration of test gas was selected by review of the chemical structure of the test article and the likely response based on other available data. Where there was no information to allow selection of a suitable starting concentration, a conservative approach was used with initial gas concentration in the region of 0.2 - 0.5% (2,000 - 5,000 ppm). If there were no positive responses, the test was repeated after at least 3 days using a higher test gas concentration until a positive response was seen. At this point, no further exposures were undertaken at this concentration as this represented the LOEL. If the difference between the LOEL and the next lowest concentration tested was large, further intermediate concentrations were tested to attempt to define the NOEL more precisely. A positive response was defined as indicated in Section 5.6 of this publication. The advantage of this test procedure is that it allows dogs with different responsiveness to adrenaline to be used while avoiding the possibility that a test material will be assigned an artificially high

NOEL or artificially low LOEL due to carrying out fixed adrenaline dose studies with groups of dogs containing very adrenaline-unresponsive dogs or very adrenaline-responsive dogs respectively.

4.3 Current protocols – Example III

Chengelis (Chengelis, 1993, 1995) developed an approach that exposed dogs to a series of escalating doses of adrenaline. At each exposure level, each animal was given a series of adrenaline injections, typically 2, 4, 6 and 8 μg/kgbw, with 2 minutes between injections. This resulted in greater consistency in treatment between animals and better survivability. It also resulted in a built-in confirmatory step in that if slight evidence of sensitisation were obtained, for example, at 4 μg/kgbw, then a more definite or greater response would be expected at 6 μg/kgbw. On the other hand, if a non-equivocal response occurred at 4 μg/kgbw, there would be no reason to proceed with exposures at higher dosages of the adrenaline challenge. Progression of the alternative exposure proceeded is shown in Table 4.3.

Table 4.3: Protocol of exposure and adrenaline challenge sequence for cardiac sensitisation

Time (min)	**Action**
Before exposure	
< 0	Animal placed in exposure apparatus. ECG electrodes connected
0	ECG recording initiated
2	Lowest dose adrenaline challenge
4	2nd level adrenaline challenge
6	3rd level adrenaline challenge
9	4th level adrenaline challenge
Exposure	
11	Exposure to gas initiated
21	Lowest dose adrenaline challenge
23	2nd level adrenaline challenge
25	3rd level adrenaline challenge
27	4th level adrenaline challenge
32	Exposure termination

5. DISCUSSION OF KEY FACTORS

When assessing the experimental design and its application to risk assessment, one needs to consider the species of animal and number used, the duration of exposure, the test material concentration, the dose of adrenaline and the response criteria.

5.1 Species

International guidelines (ICH guideline S7B, 2005), related to the selection of suitable species for conduction of safety studies with cardiovascular and cardiac electrophysiological endpoints, recognise that certain species offer distinct advantages over others. These are based on size, cardiovascular physiological parameters, hormonal and neuronal influences, and expression of cardiac ion channels. Laboratory animal species recommended for *in vivo* electrophysiological studies include the dog, monkey, swine, rabbit, ferret, and guinea-pig. The ionic mechanisms of repolarisation in rats and mice differ significantly from larger species, including humans. The primary ionic current controlling repolarisation in the rat and mouse is the transient outward current Ito, whereas the rapid and/or slow delayed rectifier, IKr and IKs, dominates repolarisation in the larger species. Therefore, as is noted in the ICH guidelines, the use of rodents to study cardiotoxic effects is not considered appropriate. This view is also supported by *in vitro* studies evaluating potential species-specific differences in drug-induced prolongation of the action potential duration and the occurrence of early after-depolarisations in isolated cardiac Purkinje fibres (Lu *et al*, 2001a). Lu and co-workers compared Purkinje fibres isolated from rabbit, dog, goat, guinea-pig, swine, and sheep heart, and concluded that rabbit and dog Purkinje fibres constitute the most sensitive models for detecting drug-induced prolongation of action potential duration and pro-arrhythmogenic effects *in vitro*.

Historically, in regard to species selection, Meek *et al* (1937) believed that the dog was more suitable than the cat, which had also been used in early cardiac sensitisation experiments. Dogs have been used for over a hundred years in studies that examine the physiological and pharmacological responses that humans might be expected to exhibit when stressed or given pharmaceutical products because dogs have been found through experience to respond in a similar manner to humans who are exposed or stressed in the same way. Additionally, the body of cardiac sensitisation data that has now been accumulated using the dog appears to give reproducible results from different investigators and the data are consistent with effects that have been observed in humans, e.g. cardiac sensitisation produced by certain volatile anaesthetic agents.

As previously noted in Section 3.1, dogs used in cardiac sensitisation studies are trained to accept a flow-through mask and to stand in a sling on a table. Suitable training of the dogs minimises

any stressful effects of the procedure. However, if the test material is irritating or if it produces central nervous system excitatory effects, one might anticipate seeing struggling in the test animals, which may compromise the conduct of the study.

The use of non-human primates in cardiac sensitisation studies has been attempted only rarely. These species are not recommended for cardiac sensitisation studies due to the difficulties involved in applying sufficient restraint to allow an accurate ECG recording and administration of the test gas whilst minimizing stress caused by the degree of restraint required.

In humans, gender has been demonstrated to constitute an independent risk factor for development of cardiac arrhythmias, particularly for Torsades de Pointes in both the congenital and acquired forms of the long-QT syndrome (Makkar *et al*, 1993; Locati *et al*, 1998; Drici and Clement, 2001; Cheng, 2006; Furukawa and Kurokawa, 2007). The heart rate-corrected QT interval is longer in females (during their reproductive phase of life) than in males, and women are at a higher risk for developing Torsades de Pointes arrhythmias than men, whereas the incidence of atrial fibrillation or sudden cardiac death in women is lower than in men (Bidoggia *et al*, 2000; Pham and Rosen, 2002; Schulze-Bahr *et al*, 2005). This gender difference in cardiac repolarisation has also been demonstrated in a variety of animal models *in vivo* as well as in *in vitro* models at various levels of complexity such as Langendorff heart, Purkinje fibres, ionic currents in isolated myocytes, and ion channel gene expression levels (rabbit: Liu *et al*, 1998, 2003; Lu *et al*, 2000, 2001b; Pham and Rosen, 2002; dog: Abi-Gerges *et al*, 2004; Fülöp *et al*, 2006; guinea pig: James *et al*, 2004; Brouillette *et al*, 2007). Based on these experimental and clinical studies, it is concluded that the gender differences in humans are small. As the specific mechanism for chemically induced cardiac sensitisation is unknown (Chapter 9), it is not possible to conclude that one sex may be more sensitive to these cardiac sensitising effects than the other.

5.2 Number of animals

The number of dogs exposed to a particular concentration of test substance used by various investigators typically ranges from 6 to 12. Some protocols specify that, following discontinuation of exposure at a given concentration when an unequivocal positive response is obtained in a single dog, no further dogs should be exposed at that concentration of test substance, or at any higher concentrations. The consensus of the authors of this document is that the use of fewer than 6 animals may result in a test that lacks sufficient statistical power, given the inherent variability in this response in both humans and dogs, and that groups of at least 6 should be exposed at each concentration of test substance not causing cardiac arrhythmias, in order to confirm that a given concentration was without effect. The protocol used by Reinhardt *et*

al (1971) generally used 6 or 12 dogs. Groups of this size have been shown to yield reproducible results (Chapter 6).

5.3 Duration of exposure

Historically, the duration of exposure to the test material has ranged from 5 to 30 minutes. Exposure of 5 minutes before the challenge dose of adrenaline is administered is often chosen to approximate to the duration of exposure time involved in "sniffing" cases. Actually, in most cases, the duration of exposure during reported episodes of "sniffing" was probably only a matter of minutes or even less. The duration of exposure to the test material may not be that critical, since later experiments have shown that the most important point is that, for a given test substance, the achievement of a threshold blood level of the test material is required. For a given concentration of the chemical, the maximum blood level is most often achieved after about 5 minutes of exposure (Table 5.3) (Mertens *et al*, 1995).

Table 5.3: Comparison of blood level, exposure period and ECG findings for dogs exposed to 1,1,1,2,3,3,3-heptafluoropropane (HFC-227ea) in two separate studies

Study	Exposure period [a] (min)	Blood concentration [b] (µg/ml)	Responders [c] (ECG grades)
1	5	35.2 ± 5.7	3/6 (≥ 3)
2	30	29.0 ± 3.2	5/10 (≥ 3)

[a] Period of time that gas was administered prior to the administration of adrenaline challenge (6 µg/kgbw)

[b] Concentration of HFC-227ea determined using head space analysis (gas chromatography, external standardisation) in whole blood drawn immediately prior to adrenaline challenge

c The number of animals showing Grade 3 or greater ECG patterns upon adrenaline challenge over the total number of animals exposed to 140,000 ppm HFC-227ea (973,000 mg/m^3) (Appendix A)

Mullin *et al* (1979) showed that arterial and venous blood levels of bromotrifluoromethane, (FC-13B1, Halon 1301) are related to the exposure levels. However, after exposure to a given concentration of the agent for about 5 minutes, the blood level reaches a plateau. The blood level of FC-13B1 drops to very low concentrations within 5 minutes after exposure is terminated.

Furthermore, when dogs were exposed to 50,000 and 75,000 ppm FC-13B1 (304,000 and 426,000 mg/m^3) for 30 minutes and 1 hour and given a challenge injection of adrenaline, 8 µg/kgbw intravenously, no arrhythmias were observed following exposure to 50,000 ppm. At 75,000 ppm, 2 out of 12 dogs exposed for 30 minutes and 1 out of 12 dogs exposed for 1 hour developed a severe cardiac arrhythmia. Two out of 12 dogs exposed to 100,000 ppm FC-13B1 (609,000 mg/m^3) for 30 minutes developed a serious arrhythmia. In tests of FC-13B1 using the standard screening protocol with a 5-minute exposure time, zero out of 62 dogs exhibited cardiac

sensitisation following exposure to 50,000 ppm, 1 out of 18 responded at 75,000 ppm, and 8 out of 69 responded at 100,000 ppm. This study supports the conclusion that the risk of cardiac sensitisation does not increase significantly at a given concentration even with prolonged exposure with this class of compounds. These comparisons are summarised in Table 5.3.

Table 5.3.: Cardiac sensitisation response as related to exposure duration and concentration of FC-13B1

Time (min)	Concentration (ppm)		
	50,000	75,000	100,000
5	0/62	1/18	8/69
30	0/12	2/12	2/12
60	0/12	1/12	-

Additional studies by Reinhardt *et al* (1973) and Mullin (1969) support this conclusion. The first showed that 10 out of 29 dogs developed serious arrhythmias when exposed to CFC-113 at a concentration of 5,000 ppm (38,300 mg/m^3) for 5 minutes and given an injection of 8 µg/kgbw of adrenaline. When a group of 12 dogs was exposed to 1,000 or 2,500 ppm CFC-113 (7,660 or 19,200 mg/m^3) for 5 minutes and then given an injection of adrenaline, no arrhythmias were seen. The second study showed that even when 6 dogs were exposed to 2,000 ppm (15,300 mg/m^3) for 6 hours, only 1 developed an arrhythmia when given an injection of adrenaline. In addition, when a group of 6 dogs was given 5 daily 6-hour exposures to 1,000 ppm CFC-113 and then injected with 8 µg/kgbw adrenaline at the end of each day, no arrhythmias occurred. One out of 6 dogs exposed to 2,000 ppm using the same protocol responded only on the first 2 days.

Experiments conducted by Azar *et al* (1973) and Trochimowicz *et al* (1974) showed that critical blood and tissue levels of test material were often associated with cardiac sensitisation. Arterial and venous blood concentrations of CFC-113 in beagle dogs during and after 10-minute exposures to 3 inspired concentrations increase rapidly during the first 5 minutes of exposure. At the end of the exposure, the blood level declined rapidly during the first few minutes followed by a slower decline. Although a wide range of inspired concentrations; e.g. 5,000 ppm CFC-113 (38,300 mg/m^3), 25,000 ppm 2-dichlorotetrafluoroethane (CFC-114) (175,000 mg/m^3), and 150,000 ppm CFC-115 (947,000 mg/m^3), is required to produce cardiac sensitisation, the arterial blood concentrations associated with these threshold sensitising levels were similar for all three chlorofluorocarbons, 12.5, 13.8 and 5.8 µg/ml blood (67, 81, 38 µmol/l), respectively. These observations might support the hypothesis that the differences in potency observed between some of the known cardiac sensitisers are due to differences in their toxicokinetics and not their

toxicodynamics. Whilst this may be a possibility, it is concluded that there are insufficient data available to test the validity of the hypothesis.

In another series of experiments, Trochimowicz *et al* (1974), with CFC-11 and CFC-12, dogs were exposed to various concentrations for 5 minutes, sacrificed immediately and chlorofluorocarbon concentrations were measured in about 10 different tissues. Tissue concentration was directly correlated with blood and inhaled concentration associated with cardiac sensitisation. In addition, no evidence of accumulation or retention of the test materials was found in tissues after acute inhalation.

5.4 Test material concentration

As indicated in Section 3.5, the selection of test material exposure levels and the order in which the exposures are carried out can influence the results of this type of study. However to avoid potentially misleading results the authors of this document recommend that one consider the ultimate application(s) of the material and what may already be known about the compound or related products. The magnitude of difference between exposure levels also needs to be given consideration since extrapolation of a true effect level between a NOEL and LOEL cannot be made with certainty.

5.5 Selection of the dose of adrenaline

The dose of adrenaline and its rate of administration are key factors in the conduct of cardiac sensitisation studies, since adrenaline dosage plays a direct role in the outcome of the study results along with the test agent exposure concentration and duration of exposure.

The maximum level of adrenaline secreted in humans during stress is reported to be around 4 - 5 µg/kgbw/min (Starling and Evans, 1962, Cannon, 1919). The dose of adrenaline used by most investigators using the protocols described above ranges from 4 to 65 µg/kgbw given intravenously over a period of as quickly as possible up to 50 seconds (Hermann and Vial, 1935; Meek *et al*, 1937; Chenoweth, 1946; Phillips *et al*, 1946; Krantz *et al*, 1948; Raventos, 1956; Bamforth *et al*, 1961). The dose used in the studies reported by Reinhardt *et al* (1971) of 8 µg/kgbw provides about 50 µg/kgbw/min. Consequently, this dose of adrenaline given exogenously is about 10 times the amount secreted by humans under stress and, thus, the model appears to be more rigorous when compared to the amount of adrenaline humans might secrete in times of extreme stress (Bridle, 1983). Therefore, one might expect that a greater concentration of a sensitizing chemical would be required to produce heart arrhythmias in humans as compared to dogs under the conditions of the protocol used by most investigators. This difference provides a built in degree of safety when extrapolating the results of these studies in the dog to humans.

Further support of this statement is provided in an experiment reported by Mullin *et al* (1972) in which beagle dogs trained to run on a treadmill to increase their levels of circulating adrenaline were exposed for 20 minutes to either CFC-11, CFC-12 or CFC-114. One out of 6 dogs showed evidence of cardiac sensitisation when exposed to 100,000 ppm CFC-12 (494,000 mg/m^3) and 1 out of 7 dogs responded at 50,000 and 100,000 ppm CFC-114 (349,000 and 699,000 mg/m^3). These effect levels were twice the effect levels observed in the exogenous adrenaline experiments. Thus, a wide margin of safety appears to exist when considering the risk encountered under stress involving the endogenous secretion of adrenaline.

In another study that examined the effects of endogenous adrenaline, dogs were exposed to CFC-113 at a level of 12,000 ppm (91,900 mg/m^3) and frightened by either a loud noise or an electric shock. No arrhythmias were seen. In another series of studies, dogs were exposed to 20,000 ppm CFC-113 (153,000 mg/m^3) while running on a treadmill (up to 300 feet/minute for 20 minutes). Again, no cardiac arrhythmias suggestive of cardiac sensitisation from CFC-113 were seen (Mullin *et al*, 1969). Reinhardt *et al* (1971) reported similar findings earlier in studies using stress such as noise to examine the threshold level for cardiac sensitisation caused by the endogenous secretion of adrenaline. The compounds studied were CFC-11, CFC-12, CFC-114, and 1-chloro-1,1-difluoroethane (HCFC-142b). The dogs were exposed to 800,000 ppm compound (4,490,000, 3,950,000, 5,590,000, 3,290,000 mg/m^3, respectively) and 20% oxygen (200,000 ppm) for 30 seconds along with a loud noise consisting of sirens, gongs, and jet takeoffs. Evidence of cardiac sensitisation was seen with all the compounds except CFC-12. The most arrhythmias were observed with HCFC-142b. The animals convulsed and or struggled a great deal and, as a consequence, the endogenous levels of adrenaline were probably higher than they would have been if the animals had not struggled. Exposure to test compound alone and or to noise alone each produced fewer responses than exposure to the two together.

Since aerosol "sniffing" involves the deliberate, deep inhalation of concentrated gases or vapours, one can see that very high concentrations of an aerosol propellant could be inhaled and produce cardiac sensitisation in an environment of excitement and/or exercise. The dose of adrenaline, 8 µg/kgbw in 1 ml of normal saline, that was selected for use in the cardiac sensitisation tests reported in the seminal work of Reinhardt *et al* (1971) was administered at a constant rate over 9 seconds. While this dose of adrenaline has been used in several studies by other researchers, it is not universally adopted. Literature reports of cardiac sensitisation studies indicate that exogenous adrenaline doses have ranged from 2 to 65 µg/kgbw administered over 10 to 40 seconds (Beck *et al*, 1973). Additionally, as noted above, cardiac sensitisation in dogs exposed to CFC-12 and CFC-114 has been demonstrated using exercise to raise the levels of endogenous adrenaline (Mullin *et al*, 1972) with no administration of exogenous adrenaline. Studies have been undertaken in which an increasing dose of adrenaline has been used. In the first of these, an appropriate adrenaline dose is selected for each dog (1–12 µg/kgbw administered in solution at 0.1 ml/kgbw of an appropriate dilution at approximately 0.1 ml/s) and, in the second, a

sequence of adrenaline doses (2, 4, 6 and 8 µg/kgbw) with 2 minutes between each injection is used. The appropriate adrenaline dose for use in these studies has been considered by Mullin *et al* (1972) and Brock *et al* (2003) who have both concluded that the commonly used regime of 8 µg/kgbw delivered over a 9 second period provides a circulating dose of adrenaline that is approximately 10 times higher than that of humans during times of stress. In view of the profound effect of the adrenaline dose on the LOEL of the test material and taking into account the safety factor provided by a dose of 5 to 10 µg/kgbw adrenaline delivered at an appropriate rate and the fact that the work of Hardy *et al* (1994) suggests that this dose may be too high for some animals, the authors of this document propose that a standard adrenaline dosing procedure should be adopted for such tests. It is concluded that the titration of individual animals to the dose of adrenaline or the giving of increasing doses of adrenaline is the preferred approach, as opposed to the administration of a fixed dose of adrenaline to every dog. In practice, however, a dose of 8 µg/kgbw (delivered over approximately 10 s) has proven to give results similar to those when differing doses are given, especially when a maximum dose of 12 µg/kgbw is not exceeded.

5.6 Response criteria – What constitutes a positive response?

The endpoint for all current cardiac sensitisation protocols is the detection of a positive response following adrenaline administration during exposure to the test substance. Therefore the criteria used for assignment of a positive response must be clearly defined and consistently applied. Review of cardiac sensitisation studies indicates that the consensus is that mild effects such as a minor increase in the number of unifocal ectopic beats in response to adrenaline challenge during test substance exposure is not sufficient evidence of cardiac sensitisation. A more significant and potentially life-threatening cardiac event such as the occurrence of multifocal ectopic activity, marked ventricular tachycardia or ventricular fibrillation is required.

5.6.1 Criteria used in dog studies

In the Reinhardt *et al* (1971) paper a ‘mild’ response was defined as “an increase in the isolated abnormal beats (abnormal complexes) and a ‘marked response’ was defined as “an arrhythmia […] which was considered to pose a serious threat to life (multiple consecutive ventricular beats) or which ended in cardiac arrest (ventricular fibrillation).” This publication also stated that “only marked responses were considered to represent a cardiac sensitizing effect”. Beck *et al* (1973) state that in their studies “Cardiac sensitization was deemed to have occurred when ventricular tachycardia or ventricular fibrillation resulted […]. An increased number of ventricular ectopic beats was not regarded as evidence of sensitisation since they could often be produced by a challenge injection of adrenaline [adrenaline] during control air exposures.” Hardy *et al* (1994) defined a positive response as “any burst of multifocal ventricular ectopic activity or ventricular

fibrillation" but have pointed out elsewhere (unpublished protocols) that knowledge of the previous ECG history of each particular dog is of great importance and should be taken into account in interpreting the ECG traces. For example, in some circumstances, for a dog known to be normally unresponsive to adrenaline even at a dose of 12 µg/kgbw a sequence of unifocal ectopic beats might be regarded as a positive response. This response is regarded as particularly important because it is known that beagle dogs have a background incidence of spontaneous arrhythmia and conduction disturbances. It is also known that the incidence of the background cardiac disturbance can vary in different groups of dogs. For example, in a study of control animal ECG data in dogs from different suppliers, ectopic beats were seen in 10 of 1,406 beagles (1/140). In a subgroup of these, which was obtained from one of the suppliers, ectopic beats were seen in 5 of 519 beagles (1/100), while in the remaining 895 dogs from the other supplier, only 6 presented ectopic beasts (Detweiler, 1981). A further definition of positive response has been proposed by Chengelis (no date) in which the severity of the response is graded on a scale of 1 (a single premature ventricular contraction in 10 s) through to 5 (very severe or potentially fatal response – fibrillation or flutter). In this scheme, responses of severity 1 to 2 would not be considered indicative of cardiac sensitisation whereas response grades 4 and 5 would. Grade 3 responses are considered equivocal evidence of cardiac sensitisation. Prior knowledge of the cardiac performance of the animal, data from the entire ECG record collected during the study (not just the short snippet shown in the examples) and other factors, e.g. behaviour, must also be considered. In studies on 1,1,1,2,3,3,3-heptafluoropropane (HFC-227ea), grade 3 responses were considered positive indicators of cardiac sensitisation. In the escalating adrenaline dose scenario used in that study, if a grade 3 was obtained at a lower dose of adrenaline, it was confirmed with the next higher adrenaline dose. Grade 3 responses are not fatal. They are unlikely to occur in dogs that are healthy and normal, and have only been reported to occur following exposure to test substance and adrenaline (Appendix D).

5.6.2 Comparison of dog criteria to human definition

As mentioned above, the more serious cardiac arrhythmias are required to be seen in the dog studies to indicate a cardiac sensitisation effect. In humans, this response would be equivalent to a higher grade tachycardic ventricular arrhythmia where the putative danger of ventricular extrasystoles in the ECG is often assessed in an approximate manner according to the classification of Lown (Lown and Wolf (1971). In general, the occurrence of isolated unifocal ventricular extrasystole (VES) in the absence of an underlying cardiac disease is apparently not associated with increased mortality, Rodstein *et al* (1971), whereas patients suffering from, e.g., coronary heart disease associated with multifocal VES, pairs, runs, or ventricular tachycardia have an increased risk for sudden cardiac death, Steinbeck (1983 and literature cited therein).

6. REPRODUCIBILITY OF FINDINGS

6.1 1,1-Dichloro-1-fluoroethane(HCFC-141b)

Three studies were conducted to measure the cardiac sensitisation potential from exposure to HCFC-141b in dogs. The first study was conducted at the Du Pont Haskell Laboratory in 1977 (Mullin, 1977). In that study, none of the 10 dogs exposed to 2,600 ppm HCFC-141b (11,100 mg/m^3) and given an injection of adrenaline (8 µg/kgbw) showed an effect; 1 of 10 exposed to 5,200 ppm (22,300 mg/m^3) showed a marked response of multiple consecutive ventricular beats of 4.5 seconds duration; at 10,200 ppm (43,700 mg/m^3), 1 of 10 developed a fatal arrhythmia and at 21,600 ppm, 2 out of 2 dogs developed fatal arrhythmias.

The second study was conducted at Huntingdon Life Science in 1989 (Brock *et al*, 1995), using 2 monkeys and 4 dogs. The data from monkeys were difficult to interpret since all exposures to both, the control substance, CFC-11, and the test substance, HCFC-141b, resulted in arrhythmias. In this study, the threshold for response in the dogs was 9,000 ppm (50,500, 38,600 mg/m^3, respectively) (1 dog showed an equivocal response at 9,000 ppm, all showed clear responses at and above 14,000 ppm [78,600, 60,000 mg/m^3, respectively] and between 9,000 and 12,000 ppm [67,400, 51,500 mg/m^3, respectively] 2 of 9 exposures resulted in responses. The dogs received multiple exposures, so the total number of exposures is greater than 4).

In the third study, also conducted at Huntingdon Life Sciences in 1994 (Kenny, 1994) using groups of 6 dogs exposed to CFC-11 and HCFC-141b. The dose of adrenaline was adjusted for each dog to achieve maximum sensitivity. The dose of adrenaline varied from 2 to 12 µg/kgbw. No dogs responded at 10,000 ppm (56,200, 42,900 mg/m^3, respectively) and 1 of 10 responded at 20,000 ppm (112,000, 85,800 mg/m^3, respectively).

Taken together, two studies predicted a clear effect of HCFC-141b at 10,000 ppm and above, while the third study predicted a clear effect at 20,000 ppm but not at 10,000 ppm. All predicted similar effects within a range of 10,000 to 20,000 ppm.

Using a markedly different protocol, Eger (1993, 1994) and Laster *et al* (1990) studied the potential of HCFC-141b to produce cardiac arrhythmias in the rat. In this study, 3 times the dose of adrenaline needed to produce cardiac arrhythmias during exposure to halothane (exposure level not provided) was administered to the rats during exposure to HCFC-141b. Exposure at 10,000 ppm (42,900 mg/m^3) produced arrhythmias (3 or more consecutive PVCs) in 13 of 15 rats and all rats were positive at 20,000 ppm. While 3 of 11 rats exposed to 5,000 ppm (21,400 mg/m^3) also showed a positive response, the object of the study was to define a median effective concentration (EC_{50}). It was concluded that exposure to 10,000 ppm HCFC-141b

combined with 3 times the halothane arrhythmogenic dose of adrenaline produced a positive response.

Even though the group sizes were small, taken together, these four studies predict a cardiac sensitisation response in both dogs and rats given injections of adrenaline when exposed to levels of HCFC-141b in the range of 10,000 to 20,000 ppm.

6.2 1,1,1,2-Tetrafluoroethane (HFC-134a)

Two studies have been conducted with HFC-134a. In the first (Mullin *et al*, 1979), dogs were exposed to levels of 50,000, 75,000 and 100,000 ppm HFC-134a (208,000, 313,000, 417,000 mg/m^3) and given an injection of 8 µg/kgbw of adrenaline. None of the 10 dogs exposed to 50,000 ppm developed an arrhythmia. Two of 10 dogs exposed to 75,000 ppm and 2 of 4 dogs exposed to 100,000 ppm exhibited a marked cardiac arrhythmia. One dog exposed to 100,000 ppm HFC-134a (417,000 mg/m^3) developed ventricular fibrillation and died. In the second study (Hardy *et al*, 1991), dogs were exposed to 40,000, 80,000, 160,000 or 320,000 ppm HFC-134a (167,000, 334,000, 667,000, 1,330,000 mg/m^3). They were also then given an injection of 8 µg/kgbw of adrenaline. No responses were seen at 40,000 ppm. At 80,000 ppm, 3 of 10 responded; at 160,000 ppm 4 of 10 responded and at 320,000 ppm 3 of 4 responded. Thus both studies showed virtually the same NOEL (40,000 - 50,000 ppm) and LOEL (75,000 - 80,000 ppm).

Alexander and Libretto (1995) conducted studies involving exposure of dogs to HFC-134a. In one phase of the work, dogs were exposed to levels of 40,000, 80,000, 160,000 and 320,000 ppm HFC-134a (167,000, 334,000, 667,000, 1,330,000 mg/m^3) for up to 1 hour. These dogs were not given injections of adrenaline. No effects were seen in the dogs exposed to 40,000 or 80,000 ppm. Exposure to 160,000 ppm HFC-134a caused salivation, head shaking and struggling in 3 out of 4 dogs. These symptoms were more severe in dogs exposed to 320,000 ppm HFC-134a and only one dog completed the exposure. Whilst some dogs poorly tolerated exposure to HFC-134a at these concentrations, none developed an arrhythmia. The same effects were seen in the exposure to 320,000 ppm and only one dog completed the exposure. However, none developed an arrhythmia. Comparing the two studies with injections of adrenaline to the study without adrenaline, the effect of the adrenalin is clear. No responses were seen at 320,000 ppm, 4 times the level where responses were seen with adrenaline injections.

7. PHARMACOKINETICS

7.1 Physiologically-based biokinetic modelling and risk assessment considerations

7.1.1 Introduction

Cardiac sensitisation is a toxicological endpoint that is frequently used to determine the human health risk associated with acute inhalation exposure to halogenated hydrocarbons and other chemicals with cardiac sensitisation potential, Reinhardt *et al* (1971). Human cardiac sensitisation risk assessment activities or determinations of safe exposure level are based fundamentally on two approaches. One approach is based on the direct application of the NOEL and LOEL exposure concentrations generated in cardiac sensitisation studies. The other approach is based on the interaction between human biological processes and the chemical of interest. The latter approach is often referred to as the physiologically-based pharmacokinetic (PBPK) modelling approach or, more descriptively, the physiologically-based biokinetic (PBBK) model .

Prior to describing the application of PBBK modelling techniques to set safe exposure levels for substances with cardiac sensitisation potential, discussion of the biokinetic behaviour of halogenated hydrocarbons used in fire suppression and refrigeration may be useful. Amongst these substances is a group of highly volatile substances that, for the purposes of this review, will be called highly volatile halogenated hydrocarbons (HVHHs). As with all volatile substances, HVHHs enter the human system via inhalation and subsequently are subject to uptake into the arterial blood. However, HVHHs equilibrate very quickly and, within a short period of time (less than 5 minutes), reach a constant concentration (steady-state) in arterial blood. The extent of the uptake of an HVHH into blood is described by its blood-air partition coefficient. Once into the arterial blood, HVHHs are transported to the body tissues where they are distributed between the blood and tissues. The extent of distribution to tissues is described by the blood-tissue partition coefficients. After HVHHs enter the body tissues, they may either passively distribute back into blood following a concentration gradient, or be metabolised by enzymes located in the tissues. HVHH metabolism occurs primarily in the liver, but many of the HVHHs of interest are either very slowly metabolised or are not metabolised at all in the human body. Metabolism generally creates a more water-soluble product, which is then removed from the body via the urine. The HVHH that is not metabolised is eliminated via exhalation. A properly parameterised PBBK human model can be used to track the extent and rate of HVHH interaction with, and movement through, the human body.

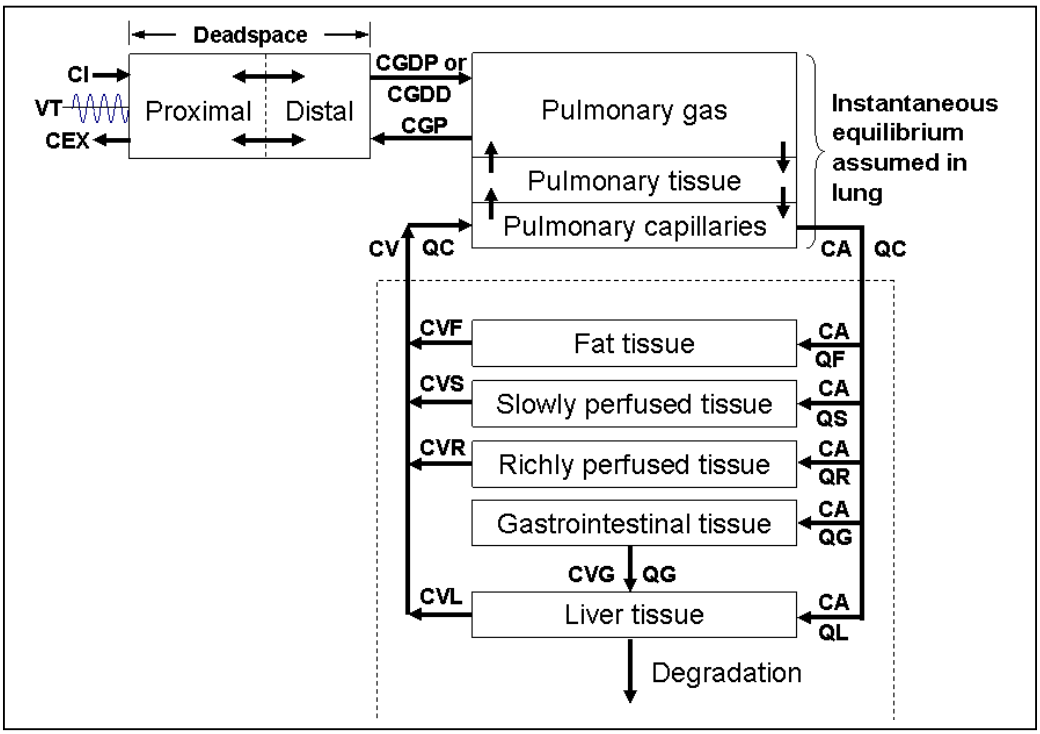
7.1.2 Model construction and data requirement

In order to construct a PBBK model that appropriately describes the interaction between the HVHH of interest and the human system, certain physiological and chemical specific data must be obtained (Andersen *et al*, 1995; Clewell and Andersen, 1995; Vinegar *et al*, 1998). Physiological data requirements include items such as breathing rate, blood flows, tissue/organ volumes, body weight and elimination mechanisms. Human physiological data are available from the medical and physiology literature. Chemical specific data requirements include blood and tissue partition coefficients, metabolic rate constants, affinity constants, the concentration of HVHH in arterial blood at the LOAEL exposure concentration, and molecular weight (Table 7.1). The generation or acquisition of chemical data often requires the use of targeted experiments. Once the data have been generated or acquired, they can be combined with the mathematical framework that describes the human system (Figure 1).

Table 7.1. PBBK model data requirements

Abbreviation	Parameter	Unit
Mr	Molecular mass	-
VmaxC	Michaelis-Menton metabolic rate, normalised to scaled bw	mg/h/kgbw
Km	Michaelis-Menton affinity constant	mg/l
Partition coefficients		
PB	Blood-air (human)	-
PFA	Fat-air (rat)	-
PLA	Liver-air (rat)	-
PRA	Richly perfused tissue-air (rat)	-
PSA	Slowly perfused tissue-air (rat)	-
Arterial blood level associated with cardiac sensitisation		
CS Val	Concentration in arterial blood at LOAEL	mg/l
Tissue volumes		
VFC	Volume of fat as a fraction of bw	-
VGC	Volume of gastro-intestinal tract as a fraction of bw	-
VLC	Volume of liver as a fraction of bw	-
VRC	Fractional volume of rapidly perfused tissue	-
VSC	Fractional volume of slowly perfused tissue	-
VGD	Dead space volume	l
Circulatory system		
QC	Cardiac output	l/h
QFC	Fractional cardiac output to fat	-
QGC	Fractional cardiac output to gastro-intestinal tract	-
QLC	Fractional cardiac output to liver	-
QRC	Fractional cardiac output to rapidly perfused tissue	-
QSC	Fractional cardiac output to slowly perfused tissue	-
Pulmonary system		
VentLM	Alveolar ventilation	l/min
VFRC	Volume of functional reserve capacity	l
VcapP	Volume of pulmonary capillaries	l
VTP	Volume of pulmonary tissue	l

Figure 1: Schematic diagram of a human breath-by-breath PBBK model[a]



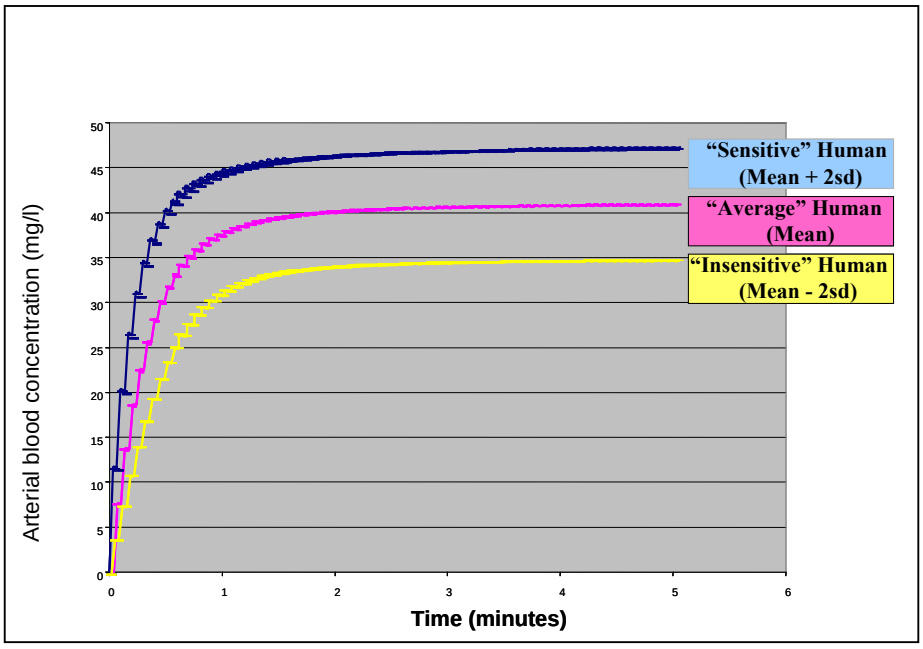
[a] The section outside of the dotted line represents the human respiratory tract. Abbreviations: VT, tidal volume; CA, CEX, CI and CV, arterial, exhaled, inhaled and venous concentrations, respectively; CVF, CVG, CVL, CVS and CVR, fat, gastrointestinal, liver, slowly perfused and rapidly perfused tissue venous concentrations, respectively; CGDD, CGDP, CGP, distal dead-space, proximal dead-space and pulmonary gas concentrations, respectively; QC, cardiac output; and QF, QG, QL, QS and QR, fat, gastrointestinal, liver, slowly perfused tissue and rapidly perfused tissue blood flows, respectively

7.1.3 Modelling of cardiac sensitisation and risk assessment in humans

Cardiac sensitisation studies are usually conducted using dogs, which are exposed to the chemical of interest in combination with an adrenaline challenge. The details of relevant protocols and the conservative nature of this test are described elsewhere in this report. A modification of the normal protocol is used to measure the HVHH concentration in arterial blood resulting from an inhalation exposure at the lowest airborne exposure concentration associated with cardiac sensitisation. The measured HVHH arterial blood concentration at the LOAEL for cardiac sensitisation is the output from the cardiac sensitisation test that is used in the PBBK modelling approach. The individual steps and a brief description of relevant elements associated with the PBBK approach are provided below.

- Generate or acquire metabolic and partition coefficients for the HVHH of interest. While rat tissue partition coefficients and metabolic constants may be used in the human model, the use of a measured human blood-air partition coefficient is important. The basis for this requirement is that the model is very sensitive to the blood-air partition coefficient, but metabolism and further distribution are not primary factors in HVHH kinetic behaviour during a 5-minute exposure.
- Generate or acquire a human PBBK model for use in simulating human inhalation of HVHHs over a 5-minute time period.
- Establish the concentration of the HVHH in dog arterial blood that is associated with the onset of cardiac sensitisation (the LOAEL). The threshold of effect blood levels for several fluorocarbons has previously been determined in whole animal experiments with dogs (Vinegar *et al*, 2000).
- Establish the HVHH concentration in human arterial blood that is associated with the onset of cardiac sensitisation. Since the HVHH level in dog arterial blood is obtained from a test that is extremely biased toward producing a cardiac sensitisation response, this level is accepted as a conservative value for humans. In order to provide even more conservatism (safety), probabilistic modelling (Monte Carlo) can be used to select a very sensitive population for use in the PBBK modelling approach. In this context, “sensitive” is defined as increased rate and extent of HVHH uptake into arterial blood during an inhalation exposure. This approach results in using a subpopulation that is approximately 2 standard deviations (sd) more sensitive than the ‘average’ population (Figure 2).

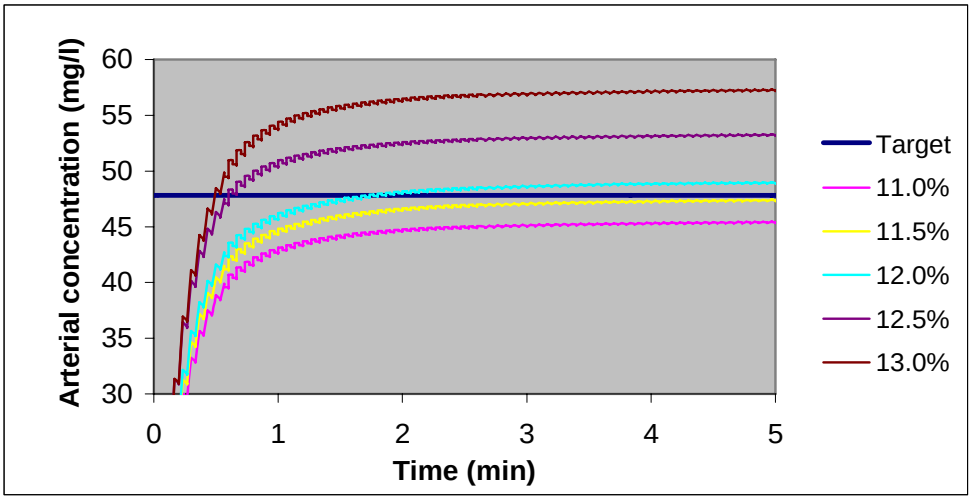
Figure 2: Monte Carlo simulation to establish a sensitive human subpopulation



- Run model simulations using the human PBBK model to determine which HVHH exposure scenarios produce an HHVH level in human blood that are associated with cardiac sensitisation.

This information can then be used to construct safe exposure-time profiles to maximise HVHH performance and protect exposed individuals from cardiac sensitisation. An example of the PBBK model output against a target (maximum safe HVHH arterial blood concentration) is shown in Figure 3. In this example, HVHH exposures of 115,000 ppm and lower would be safe with respect to the production of cardiac sensitisation, even in sensitive humans. If HVHH exposure concentrations were higher than 115,000 ppm, a shortened exposure time would be required to provide a safe environment, with respect to cardiac sensitisation. Detailed examples and the use of PBBK output in risk assessment have been published (Vinegar *et al*, 2000; NFPA, 2004).

Figure 3: Human arterial blood levels during inhalation



7.1.4 Concluding comment on PBBK model approach and its use in risk assessment

Human PBBK modelling allows the data generated in adrenaline-challenged dog cardiac sensitisation studies to be used in setting safe exposure limits that are protective against HVHH related cardiac sensitisation effects in human (Vinegar *et al*, 1999; Vinegar *et al*, 2000). The approach was created and validated as the result of the US EPA, US Department of Defense and Industry research (Vinegar *et al*, 2000). Since human physiology and chemical specific interactions are used in this approach, it helps to identify HVHH exposure concentrations that are protective for humans, which may be either higher or lower than the HVHH exposure levels associated with the threshold of cardiac sensitisation (LOAEL) in the adrenaline-challenged dog. The approach is designed to be protective of sensitive human subpopulations and is currently used by the US National Fire Protection Association (NFPA, 2004).

8. RISK ASSESSMENT

8.1 Interpretation of data

Theoretically, when one chooses to rank the cardiac sensitisation potential of chemicals, the most appropriate way is to base the ranking on the measured EC_{50} levels. Mathematically, this metric is the most relevant descriptor of potency and thus will yield the best basis for comparison. However, if the data are to be applied directly to a risk assessment for that chemical, it is often better to use the threshold or no observed effect level and then apply an uncertainty or safety factor to that value. While these values may be less well defined than the EC_{50}, one is starting from a level that defines either the lowest level that has been associated with the effect or the highest level that has been shown not to cause that effect. For the risk assessment, if the effect was seen in animal studies, one can then estimate the corresponding sensitivity of the human population at risk. This assessment will be based on:

1. The severity of the effect seen in the animal population and how this will translate to effects in humans;
2. the sensitivity of the test species;
3. the severity of the effect that would be anticipated in humans;
4. the comparative sensitivity of the test species to humans;
5. concern about sensitive human subpopulations.

A modern approach for estimation of the no-effect-level is to determine the benchmark dose or concentration (BMC) associated with a specific threshold. End points that are often considered are the BMC_{01} or the $BMCL_{05.}$ The BMC_{01} is the maximum likelihood (median) estimate for the no effect level for the test population. The $BMCL_{05}$ is the lower 95^{th} percentile estimate for the no effect level in the test population. These calculations require a robust data set. With cardiac sensitisation studies there is usually not enough data to calculate the benchmark dose points. Instead, two exposure concentrations are defined, the highest level not causing cardiac arrhythmia being taken as the NOEL and the lowest level that resulted in a cardiac arrhythmia (in less than 50% of the dogs where more than 1 dog has been tested at that exposure level) being taken as the threshold or LOEL. Dogs given the sub-arrhythmogenic doses of adrenaline are extremely sensitive to the effects of the exposure to cardiac sensitisers and therefore the effect levels have been used without application of any additional safety factor. Thus the NOEL is the highest demonstrated safe level for exposure and the LOEL is considered a conservative estimate for the threshold at or above which one might see an arrhythmia.

8.2 Human considerations

Taking into account the major protocol parameters as outlined above, a reasonable assumption would be that the experimental design used by Reinhardt *et al* (1971) provides a rigorous screening test for determining the cardiac sensitisation potential of a chemical. Human experience would bear this assumption out since no deaths have been reported from exposure to chlorofluorocarbons or hydrofluorocarbons when exposure to recommended levels has been observed. Further supporting evidence that recommended exposure levels do not pose a risk of cardiac arrhythmias comes from several studies involving human subjects.

Azar *et al* (1972) saw no evidence of cardiac arrhythmias in two subjects exposed to 1,000 and 10,000 ppm CFC-12 (4,940, 49,400 mg/m^3) for 2.5 hours. Four subjects exposed to 500 and 1,000 ppm CFC-113 (3,830, 7,660 mg/m^3) for 1 week at each concentration did not show any disturbance in heart rhythm as monitored by the pulse rate.

Kehoe (1943) reported a cardiac arrhythmia in a subject exposed to 110,000 ppm CFC-12 (544,000 mg/m^3) for about 10 minutes. Another subject exposed to 40,000 ppm (198,000 mg/m^3) for 14 minutes did not experience any cardiac arrhythmia although definite central nervous system effects were observed and the concentration of the compound was decreased to 20,000 ppm (98,800 mg/m^3) for the remainder of the 80-minute exposure. As mentioned in Section 2.1.3, several clinical studies of refrigeration and aerospace workers did not show any evidence of a work related effect on heart rhythm.

A clinical study was conducted in which both HFC-134a and HFC-227ea vapours were separately administered (1 h/wk, whole-body exposure) for 8 weeks to healthy volunteers (4 male, 4 female) by inhalation first to air alone and then during the succeeding weeks to ascending concentrations of 1,000, 2,000, 4,000 and 8,000 ppm of either HFC-134a (4,170, 8,340, 16,700, 33,400 mg/m^3) or HFC-227ea (6,950, 13,900, 27,800, 55,600 mg/m^3) interspersed with a second air exposure and two exposures to CFC-12 at 1,000 and 4,000 ppm (4,940, 19,800 mg/m^3). Blood pressure, blood levels of the hydrofluorocarbons, pulse, cardiac function, respiratory function and effects on the central nervous system were measured and evaluated. There were no observed adverse effects, no evidence of effects on the central nervous system, and no symptoms of upper respiratory tract irritation. Blood concentrations increased rapidly and in an exposure-dependent manner. They approached steady state within 30 minutes and tended to be higher in males than females. Following the end of the exposure period, blood concentrations declined rapidly, predominantly biphasically and independent of exposure concentration. The mean residence time was 44 minutes. It was concluded that, under these test conditions, human exposure to HFC-134a or HFC-227ea did not result in any adverse effects on pulse, blood pressure, electrocardiogram or lung function, even at levels up to 8,000 ppm for 1 hour (Emmen *et al*, 2000; CoR 1a).

8.3 Other factors

An experiment was carried out using dogs that had artificially produced myocardial infarctions verified by clinical observations such as blood enzyme and ECG changes and subsequently pathological examination. These animals, upon recovery, were exposed to 1,1,1-trichloroethane, CFC-11 or FC-13B1, which had been tested previously using the screening protocol described in Section 4.1. No evidence of increased cardiac sensitivity was observed which suggests that persons who have had heart attacks are not at greater risk of developing cardiac arrhythmias when exposed to cardiac sensitising agents, Trochimowicz *et al* (1976). Reinhardt *et al* (1971) investigated a possible role for anoxia in cardiac sensitisation induced by either CFC-12 or octafluorocyclobutane (FC-C318). One of 6 dogs exposed to 70,000 ppm CFC-12 (346,000 mg/m^3) and 8.0% oxygen (80,000 ppm) for 30 seconds showed a cardiac sensitisation response, while 0 of 7 dogs, similarly exposed, showed no response with a normal oxygen concentration. In the case of FC-C318, 5 of 6 dogs exposed to 500,000 ppm test compound (2,530,000 mg/m^3) and 10% oxygen (100,000 ppm) for 5 minutes exhibited cardiac sensitisation as did 5 of 6 dogs similarly exposed with oxygen enriched to 20% (200,000 ppm). The results of these experiments suggest that severe hypoxia may enhance to a slight degree the cardiac sensitisation potential of chemicals. However, in the normal study design, the oxygen level is reduced only slightly (normally not as low as 10%) and is not a significant factor in the outcome of the test. The effect of hypercarbia (hypercapnia) was not investigated in these experiments; however, Price *et al* (1958) believe that hypercarbia is an important factor in enhancing cardiac sensitisation potential.

8.4 Summary

The risk assessment process regarding cardiac sensitisation should take into account the extrapolation of the animal data to humans, individual susceptibility, the health status of the individual and other factors such as medication. The current protocols appear to provide accurate, robust scientific information relative to the cardiac sensitisation potential of chemicals of interest and are valid predictors of cardiac sensitisation potential for use in risk assessment. These protocols are designed conservatively with built in safety factors and thus no additional safety factors need to be applied in the risk assessment process. However, one should consider exploring ways that might improve the applicability and utility of the test as a risk assessment tool.

9. MECHANISM OF ACTION

9.1 Introduction

Cardiac sensitisation has long been recognised as a potentially serious human health risk during exposure to high concentrations of many halogenated and unsubstituted hydrocarbons. Therefore, new chemical entities designed to be marketed as replacements for products such as aerosol propellants, refrigerants, solvents, and fire-extinguishing agents are required to undergo extensive *in vivo* testing for risk evaluation and regulatory purposes. However, in-depth investigations in order to elucidate the underlying mechanisms of these compounds at a cellular and/or molecular level have not been conducted or published so far. The only notable exceptions are halogenated hydrocarbons used as inhalation anaesthetics in human patients where the literature is quite extensive. Therefore, the accumulated knowledge on the mechanisms of action of inhalation anaesthetics is reviewed and summarised in the remainder of this section in order to illustrate the putative mechanisms of action of this group of halocarbons (Himmel, 2009).

9.2 Cardiac sensitisation to catecholamines by volatile anaesthetics in vivo

The proclivity of general anaesthetic agents such as chloroform, cyclopropane, halothane, methoxyflurane, and enflurane to sensitise the heart to the arrhythmogenic action of various catecholamines is a well-known phenomenon (Reynolds, 1984). In addition to numerous animal studies, the sensitising activity of inhalation anaesthetics has also been investigated in several clinical studies. Appendix E summarises the available data on arrhythmogenic doses (plasma concentrations) of (nor)adrenaline in humans and dogs.

Other investigators have demonstrated that volatile anaesthetics delay ventricular repolarisation in healthy unpremedicated human subjects (Schmeling *et al*, 1991; Kuenszberg *et al*, 2000) and that this effect is potentiated in patients with congenital long QT syndrome (Gallagher *et al*, 1998). However, delayed ventricular repolarisation regardless of whether drug-induced or congenital in conjunction with catecholaminergic stimulation is an important predisposing factor in the generation of Torsades de Pointes arrhythmias (Vos *et al*, 2001). Torsades de Pointes arrhythmias could be provoked by catecholamine (dobutamine) infusion in a patient with idiopathic long QT syndrome, whereas in control subjects with normal QT interval dobutamine infusion remained without any effect (Fujikawa *et al*, 1998). Canine models of drug-induced Torsades de Pointes arrhythmias designed for drug safety evaluation have confirmed these findings by demonstrating that under conditions of delayed ventricular repolarisation halothane anaesthesia is more arrhythmogenic than isoflurane or pentobarbital anaesthesia (Weissenburger *et al*, 2000; Yamamoto *et al*, 2001).

9.3 Mechanisms of arrhythmogenesis in general

The normal electrophysiological behaviour of the heart is determined by the timely and spatially ordered propagation of excitatory stimuli that result in rapid depolarisation and slow repolarisation, thus generating action potentials in individual myocytes. Abnormalities of impulse generation, propagation, or the duration and configuration of individual cardiac action potentials form the basis of disorders of cardiac rhythm (Roden *et al*, 2002). The integrated activity of specific ionic currents generates action potentials, the configurations and durations of which vary in specific regions of the heart (e.g. atrium vs. ventricle) as well as in specific areas within those regions (e.g. epicardium vs. endocardium vs. midmyocardium vs. Purkinje cells). Such physiological heterogeneities likely reflect variations in expression or function of the repertoire of ion channels and accessory proteins that constitute cardiac ion currents. Exaggeration of these heterogeneities, e.g. by changes in heart rate, ion channels mutations, or drug exposure, may promote re-entrant excitation, a common mechanism for cardiac arrhythmias, (Roden *et al*, 2002). The acute electrophysiological response of an individual myocyte to exogenous factors, e.g. drugs, ischemia, or autonomic activation, likely reflects changes in function of individual ion channels. The action potential represents the net effect of multiple time- and voltage-dependent events; thus, one common arrhythmogenic mechanism is that alterations of one component of such a complex system result in altered behaviour of other normal components which then may generate arrhythmias. In this regard, deranged intracellular Ca^{2+} handling is of particular importance because intracellular Ca^{2+} concentration is elevated by action potential prolongation and elevated Ca^{2+} in turn directly and indirectly modulates the activity of multiple ion channels (Roden *et al*, 2002). In the past two decades, evidence has accumulated that inhalation anaesthetics interact not only with the lipid phase of cell membranes but also directly activate or inhibit ion channels that are essential for synaptic transmission in the central nervous system and for the normal electrophysiological behaviour of the heart. If anaesthetics, and possibly other halocarbons, inhibit some channel populations more than others, they will disturb the well-balanced heterogeneity of cardiac excitation and will bring about electrical instability (Hüneke *et al*, 2004). The interactions of inhalation anaesthetics and whenever possible other halocarbons with cardiac and neuronal ion channels and other targets as well are summarised in the following paragraphs.

9.4 Interaction of volatile anaesthetics with cardiac targets

9.4.1 Adrenergic and muscarinic receptor-mediated signal transduction

Cardiac sensitisation to the arrhythmogenic action of catecholamines in the presence of volatile anaesthetics is attenuated by treatment with α- and/or β-adrenergic receptor antagonists (Maze and Smith, 1983; Carceles *et al*, 1990) and may involve deranged intracellular Ca^{2+} handling

(Roden *et al*, 2002). Both adrenergic and muscarinic receptor signalling may be important in this context since both couple to the adenylate cyclase, via stimulatory G_s or inhibitory G_i proteins, and hence contribute to modulating the basal phosphorylation state of ion channels and other cellular proteins. Several groups have indeed shown that volatile anaesthetics may interfere with this finely balanced signal transduction cascade (Anthony *et al*; 1989; Böhm *et al*, 1993; Böhm *et al*, 1994). Taken together, halothane (and possibly other halocarbons) impairs the function of the inhibitory $G_{i\alpha}$ protein resulting in an increased adenylate cyclase activity, thus favouring phosphorylation of, e.g. L-type Ca^{2+} channels and potentially pro-arrhythmic intracellular Ca^{2+} overload.

9.4.2 Catecholamine reuptake

Upon stimulation of sympathetic neurons, noradrenaline and cotransmitters (adenosine triphospate and neuropeptide Y) are released. The released noradrenaline and adrenaline secreted during stress by the adrenal medulla are inactivated by neuronal and/or extraneuronal uptake. Catecholamine-induced, α_1- and β_1-adrenoceptor-mediated downstream phosphorylation-dephosphorylation events result in rapid changes of ion channels activity, i.e. calcium channel activation and potassium channel inactivation. Thus an inhibitory interaction of halocarbons with catecholamine reuptake should promote cardiac sensitisation. However, results from *in vitro* experiments are equivocal (no effect: Brown *et al*, 1972; reuptake inhibition: Tas *et al*, 1987). Another line of evidence suggests that cardiac sensitisation by faulty neuronal noradrenaline uptake might by the primary cause for the increased risk for sudden cardiac death in patients with panic disorder (Kawachi *et al*, 1994a, 1994b; Esler *et al*, 2006). In these patients, sympathetic nervous and adrenal medullary activity were increased during panic attacks, and adrenaline spill-over from the heart was elevated at rest (Wilkinson *et al*, 1998; Esler *et al*, 2004), most likely due to hyper-methylation-mediated silencing of the noradrenaline transporter gene (Esler *et al*, 2006).

9.4.3 Cardiac sodium and calcium channels

The fast sodium inward current plays an important role in impulse conduction within the heart and in generation of certain types of arrhythmia, and calcium influx across sarcolemmal membranes initiates and modulates cardiac muscle contraction. Therefore, sodium as well as calcium channels must be considered as potential targets for halocarbons in the context of cardiac sensitisation. Indeed, clinically relevant concentrations of halothane reduced sodium current amplitude, shifted the steady-state inactivation curve to more negative potentials, and delayed recovery from inactivation/block in rat ventricular myocytes (Ikemoto *et al*, 1986). Appendix F presents the potency of volatile anaesthetics at cardiac ion channels / targets . These results were confirmed for halothane and extended to other volatile anaesthetics (isoflurane, sevoflurane) and

Purkinje cells by several groups (Chou *et al*, 1991; Eskinder *et al*, 1993; Weigt *et al*, 1997; Weigt *et al*, 1997a; Stadnicka *et al*, 1999). Clinical concentrations of some volatile anaesthetics depress cardiac contractility. This is due to inhibition of cardiac L- and T-type calcium currents [Appendix F; Bosnjak *et al*, 1991; Eskinder *et al*, 1991; Hirota *et al*, 1992; Puttick and Terrar, 1992; Takahashi *et al*, 1994; Pancrazio, 1996). Hence, volatile anaesthetics interfere with pathways contributing to intracellular Ca^{2+} homeostasis. Furthermore, sodium channels are subject to modulation by both protein kinase C (PKC) and protein kinase A (PKA) (Roden *et al*, 2002). Indeed, co-expression experiments have shown that halothane reduced Na^+ current only after PKC co-expression (Patel *et al*, 2000), and volatile anaesthetics interact synergistically with the α1-adrenoceptor/PKC and β-adrenoceptor/PKA pathways to inhibit cardiac Na^+ current, (Weigt *et al*, 1997b, 1998a, 1998b). These additive inhibitory effects on Na^+ channels are expected to attenuate electrical impulse conduction, and catecholamines should promote Ca^{2+} influx even in the presence of volatile anaesthetics, so that both mechanisms facilitate pro-arrhythmia.

9.4.4 Cardiac repolarising K^+ currents: I_{Kr}, I_{Ks}, inward rectifiers, I_{to}, and two-pore-domain channels

Volatile anaesthetics have been demonstrated to delay ventricular repolarisation in healthy, unpremedicated human subjects (Schmeling *et al*, 1991; Kuenszberg *et al*, 2000) as well as to potentiate QT prolongation in subjects with congenital long QT syndrome, Gallagher *et al* (1998). The duration of the QT interval in the ECG or the action potential in an *ex vivo* situation largely depends on the activity of repolarising delayed rectifier potassium currents, the rapidly activating I_{Kr} and the slowly activating I_{Ks}. Loss of function mutations of both channels have been genetically linked to the Long QT syndrome and Torsades de Pointes arrhythmias, and both I_{Kr} and I_{Ks} are targets for volatile anaesthetics at clinically relevant concentrations *in vitro* (Correa, 1998; Chen *et al*, 2002; Suzuki *et al*, 2003; Shibata *et al*, 2004; Scholz *et al*, 2006; Yamada *et al*, 2006; Kang *et al*, 2006) and *in vivo* (Takahara *et al*, 2005a, 2005b; Appendix F). Inward rectifier K^+ currents contribute to the terminal repolarisation of the action potential and are major players in maintaining the resting membrane potential. Among the 3 types of inward rectifiers, basal current through G protein-gated GIRK1/GIRK4 channels was enhanced by low concentrations of volatile anaesthetics, whereas muscarinic agonist-induced current was inhibited, Weigl and Schreibmayer (2001). The other two types of inward rectifiers, I_{K1} and the ATP-sensitive K^+ current, were only weakly affected (Stadnicka *et al*, 2000; Fujita *et al*, 2006). The transient outward K^+ current I_{to} is confined to certain species including rat, rabbit, dog, and human and displays regional variations of expression (subepicardium > subendocardium) which results in transmural differences of action potential duration and hence refractoriness. Halothane, isoflurane, and sevoflurane were shown to inhibit I_{to} (Davies *et al*, 2000; Rithalia *et al*, 2004; Kang *et al*, 2006) resulting in a potentially pro-arrhythmic reduction of the transmural dispersion

of repolarisation/refractoriness. Two-pore-domain K^+ channels that are prime targets for volatile anaesthetics in the central nervous system, but are also expressed in cardiac tissue (TREK-1 in rat atrial myocytes: Terrenoire *et al* (2001); TASK-1 in mouse atrial myocytes: Barbuti *et al* (2002). However, the potential contribution of cardiac 2-pore-domain K^+ channels to the phenomena of cardiac sensitisation remains to be elucidated.

9.4.5 Action potentials, conduction, and intercellular coupling

As detailed above, volatile anaesthetics have been demonstrated to inhibit depolarizing sodium and calcium inward currents as well as various repolarising potassium outward currents. Since the former is expected to result in a shortening of action potential duration and effective refractory period, whereas the latter should have the opposite effect, it is important to gain a clear understanding of the net effect in various regions of the heart. Halothane shortened the action potential duration in ventricular, but not in atrial myocytes, which was explained by the greater functional importance of calcium channel block in ventricle (Hirota *et al*, 1989; Terrenoire *et al*, 2000). The study of regional differences (proximal vs. distal) of canine Purkinje fibre action potential duration showed that halothane produced concentration-dependent decreases of proximal action potential duration without affecting distal action potential duration, in a similar manner to that of low concentrations of the sodium channel blocker tetrodotoxin (Turner *et al*, 1990). Thus the combination of reduced regional action potential duration dispersion with decreased His bundle effective refractory period was concluded to contribute to facilitation of re-entry arrhythmias. An inhibition of sodium current is usually associated with a reduction of conduction velocity, and impaired impulse propagation may play a role in the development of cardiac arrhythmias. Using the dual microelectrode technique, halothane was demonstrated to impair conduction in canine Purkinje fibres as well as to slow impulse propagation across the Purkinje fibre-papillary muscle junction (Freeman and Muir, 1991a), and to decrease effective refractory period and action potential duration in Purkinje fibres (Freeman and Muir, 1991b). In the latter study, α_1-adrenoceptor stimulation restored effective refractory period and action potential duration in Purkinje fibres exposed to halothane, but also aggravated the halothane-induced triangulation of the action potential, a pro-arrhythmia marker. These findings were confirmed and extended by subsequent studies (Turner *et al*, 1993a, 1993b; Vodanovic *et al*, 1997; Kulier *et al*, 2000). However, since the myocardium is an electrical syncytium (cells fused together), conduction velocity is not only determined by the availability of Na^+ channels, but also by a functional electrical cell-to-cell coupling via gap junction channels. Indeed, there is some evidence for an inhibitory influence of general anaesthetics on intercellular coupling, albeit at supratherapeutic concentrations (Terrar and Victory, 1988; Niggli *et al*, 1989).

9.5 Summary of mechanisms contributing to cardiac sensitisation by volatile anaesthetics

In summary, volatile anaesthetics appear to interact with cardiac ion channels at the following approximate multiples of the minimal alveolar anaesthetic concentration (MAC) (Appendix F): sodium (> 2), calcium (–30% at > 2), human ether-a-go-go related gene (hERG) (–20% at > 1), I_{Ks} (–20% at > 1), I_{to} (> 2), inwardly rectifying potassium $K_{ir}2.x$ (> 6), $K_{ir}3.x$ (1-2), action potential duration / effective refractory period (Δ10% at >2 MAC, triangulation), conduction (> 2), intercellular coupling (≈ 4). Therefore, the available experimental evidence suggests that physiologically relevant interactions of volatile anaesthetics at concentrations of 1 to 2 × MAC occur predominantly with the main repolarising cardiac potassium channels hERG and I_{Ks}. At slightly higher concentrations, i.e. at ≥ 2 × MAC, calcium and sodium channels become involved too. Altogether, subsequent alterations of the action potential duration and shape (triangulation), effective refractory period, and impulse conduction follow, and thus physiologic regional heterogeneity is likely to become exaggerated and may promote re-entrant excitation as a common mechanism for cardiac arrhythmias. From a theoretical point of view, triangulation, i.e. slowing of repolarisation and slowing of conduction, are the two most pro-arrhythmic changes in the cardiac action potential (Hondeghem, 2007). The former, triangulation, allows for reactivation of calcium current, more time for Na-Ca exchange current, reactivation of sodium current, reduced synchronisation of action potentials, and facilitation of re-excitation to generate early after-depolarisations (early after-depolarisationsGrant and Tranquillo, 2007). The latter, conduction, is an important parameter in the product of conduction velocity times refractory period λ, which determines whether re-entry is impossible (λ > available length of excitable tissue) or facilitated (λ < available length of excitable tissue). Particularly when λ is shortened in combination with triangulation, the heart almost always goes into ventricular fibrillation, and sudden cardiac death ensues. Concomitantly, voltage-clamp experiments in guinea pig and rabbit ventricular myocytes showed that action potential triangulation accelerates L-type calcium channel recovery from inactivation, leading to instability of the cell membrane potential during repolarisation, which could result in early after-depolarisations, triggered activity, and Torsades de Pointes arrhythmias (Guo *et al*, 2007). Similar experiments in repolarisation reserve-reduced rabbit Langendorff hearts demonstrated that the propensity of an individual heart to develop a Torsades de Pointes arrhythmia is high in the presence of pronounced dispersion of repolarisation and triangulation of the action potential, (Milberg *et al*, 2007). Hence, volatile anaesthetics and possibly also other halocarbons affect the cardiac electrophysiology (action potential triangulation, slowing of conduction) in a manner that should facilitate the generation of arrhythmias. If such a sensitised heart is then challenged with endogenous or exogenous catecholamines, slowing of conduction and triangulation is potentiated as demonstrated experimentally (Freeman and Muir, 1991b; Vodanovic *et al*, 1997; Kulier *et al*, 2000) and catecholamine-induced activation of a probably already disinhibited adenylate cyclase, (Böhm *et al*, 1994), results in stimulation of L-type calcium current, thus probably initiating early rly after-depolarisations and triggering an arrhythmia. Furthermore, normally silent loss-of-function

mutations in cardiac ion channel genes, a faulty catecholamine reuptake, and/or certain cardiovascular disease states such as left ventricular hypertrophy and heart failure where action potential triangulation is commonly observed, might characterise those human subjects with a particularly high risk to experience sudden cardiac death due to sensitisation of the heart to catecholamines in the presence of halocarbons and unsubstituted hydrocarbons.

In summary, cardiac sensitisation is clearly a complex event involving multiple possible mechanisms. Exactly which mechanism(s) is/are targeted by these substances is not fully understood at present.

10. ALTERNATIVE APPROACHES

10.1 Political and regulatory environment

The current political environment in most of the industrialised countries is characterised by increasingly strict regulations regarding animal experimentation and animal welfare, and favours the so-called 3Rs (replacement, refinement and reduction of animals in research) principle. In addition to the ethical and political considerations, the relatively high costs for the use of experimental animals and the need for a refined test system could lead to the development of an alternative method for the evaluation of the cardiac sensitisation effects of new chemical substances.

Nowadays, the assessment of possible cardiotoxic potential of new drugs is a routine test for the pharmaceutical industry. In particular, screening studies for the evaluation of anti- or pro-arrhythmic effects are carried out in the early phase of a drug's pre-clinical development, although *in vivo* data from relevant animal models, e.g. the dog, are the preferred basis for the risk assessment of cardiotoxic potential of human pharmaceuticals (ICH guideline S7B, 2005).

The *ex vivo* or *in vitro* techniques currently adopted for screening purposes in the safety pharmacology area may be used for the development of alternative testing methods for the assessment of the cardiac sensitisation potential of industrial chemicals. While the assessment of cardiac sensitisation potential of halogenated hydrocarbons will, for the foreseeable future, probably continue to require final experiments in dogs or another relevant species, e.g. minipigs, the antecedent steps during development, could possibly be addressed by combining various biochemical and cell-based *in vitro* assays as well as *ex/in vivo* models as outlined below.

10.2 Biochemical in vitro assays and in silico structure-activity prediction

Inhalation anaesthetics (halothane, methoxyflurane, chloroform), alcohols, ketones, ethers, alkanes have been demonstrated to be able to inhibit the activity of pure firefly luciferase at anaesthetic concentrations in a competitive manner with the binding site accommodating 1 large or two small molecules (Franks and Lieb, 1984). This also holds true for rat brain synaptosomes where saturable binding of ^{14}C-halothane with low affinity (Kd $\approx$ 0.49 mM) and partial binding inhibition by isoflurane, chloroform, FC-13B1, and dichlorotrifluoroethane (HCFC-123) has been reported (El-Maghrabi *et al*, 1992). Further studies suggested that the aqueous potency of a molecule as a general anaesthetic or an inhibitor of luciferase is determined mainly by its size (which increases potency) and its ability to accept a hydrogen bond (which decreases potency), but only marginally by its ability to donate a hydrogen bond or by its dipolarity and polarisability, (Abraham *et al*, 1991). The importance of hydrogen bonds was also emphasised by a study

showing short chain alcohol interaction with lipid membranes at the phosphate moiety to weaken the membrane-water interaction (Chiou *et al*, 1992). Two other groups observed that both polarity and steric factors are important molecular parameters to determine anaesthetic potency of fluorinated versus non-fluorinated molecules (Eger *et al*, 1999; Ueno *et al*, 1999).

Despite all this previous work, the exact molecular nature of the anaesthetic binding site(s) at the firefly luciferase molecule and at cardiac ion channels is unknown, and therefore *in silico* structure-activity prediction of the cardiac sensitisation potential of halogenated hydrocarbons does not appear to be feasible at present. On the other hand, inhibition of luciferase activity has been shown to correlate very well with anaesthetic potency. Thus, with a small experimental effort one should be able to determine whether halogenated hydrocarbons inhibit luciferase activity too, and whether their inhibitory potency in the luciferase model correlates with their cardiac sensitisation potential in dogs.

10.3 Cell- and tissue-culture-based in vitro and ex vivo models

Primary cultures of cardiac myocytes are another test system currently used for the screening assessment of cardiac effects in safety pharmacology. The culture of cardiomyocytes is generally carried out from neonatal rats with experimental methods based on the original work of Harary and Farley (1963). According to this protocol, the heart is collected from 2 to 3 days old rats and digested via mechanical and enzymatic treatment. Further treatments are necessary for the separation of cardiomyocytes from the non-muscular cell populations (Chlopcikova *et al*, 2001). When maintained in culture, the neonatal rat cardiomyocytes exhibit spontaneous synchronous beating activity for a relatively long period of about 40 days (Fu *et al*, 2005). Although neonate rats are the preferred source, methods for the preparation of cardiac myocyte cultures are reported also for other species, such as mouse, guinea pig, rabbit and chick (Chlopcikova *et al*, 2001). Beside the isolation from embryonic, neonatal and adult animals, cardiomyocytes derived from mouse embryonic stem cells were able to differentiate into beating cardiomyocyte clusters (Reppel *et al*, 2007).

An early approach to the use of heart cell cultures as a tool for the evaluation of cardiac sensitisation potential was described by Miletich *et al* (1983). They exposed spontaneously beating myocardial cells cultured from neonatal rats to various concentrations of halothane or enflurane either alone or in combination with adrenaline and demonstrated by means of video image analysis a concentration-dependent progressively increasing incidence of culture plates displaying arrhythmia. More than two decades later, this approach was used again with neonatal rat cardiomyocytes cultured on multi-electrode arrays in order to investigate cardiac sensitisation of FC-13B1 by measuring and analysing field potentials (Jiao *et al*, 2006). Interestingly, the protocol to treat the cell cultures with adrenaline and the reference compound FC-13B1 was

reported to be based on the method developed by Reinhardt *et al* (1971) (Section 2). While adrenaline alone induced only an increase in the beating rate, the simultaneous exposure to FC-13B1 and adrenaline opposed the effect of adrenaline alone on beating rate and caused a decrease in conduction velocity. Although the authors remarked that a clear arrhythmic effect of FC-13B1 could not be demonstrated in these experiments, the study could be a good base for the development of an alternative *in vitro* test for the assessment of cardiac sensitisation.

The multi-electrode array technique consists of a matrix of extracellular microelectrodes placed in a bidimensional grid layout and inserted in a support embedded in the floor of cell culture dishes, for a description see Offenhäusser and Knoll (2001) and Stett *et al* (2003). In contrast to video image analysis of contractions, the field potentials detected by means of multi-electrode array allow a partial reconstruction of the shape and time course of the underlying action potentials. Furthermore, the multi-electrode array system allows for investigation of electrical impulse conduction parameters, even including programmed electrical stimulation, interventions by means of pharmacological tools, and it could be run in parallel in order to achieve a higher throughput. multi-electrode arrays have been applied in the field of cardiac safety pharmacology using embryonic chick heart cells to detect field potential prolongation by E-4031, amiodarone, quinidine, and sotalol (Meyer *et al*, 2004). However, multi-electrode arrays could also help to detect adverse electrophysiological effects of drugs in cultured embryonic stem-cell-derived cardiomyocytes (Hescheler *et al*, 2004; Reppel *et al*, 2007) and other cardiac cell lines, e.g. H9c2 cells (Zordoky and El-Kadi, 2007) as well as in, probably more physiological, *ex vivo* ventricular slice preparations (Pillekamp *et al*, 2006).

Another advantage of the multi-electrode array system is its potential to investigate adverse neuropharmacological drug effects, either in classical slice preparations such as the adult rat hippocampal slice (Steidl *et al*, 2006) or in cultured rat cortical neurons (Xiang *et al*, 2007).

10.4 Ex vivo pro-arrhythmia models

10.4.1 Isolated perfused heart (Langendorff heart model)

The method for the isolation and perfusion of cat, dog or rabbit heart was described for the first time by Langendorff (1895). This *ex vivo* model has become a milestone for the assessment of cardiotoxic potential of drugs and offers a broad spectrum of potential measurements, high reproducibility and relatively low cost in comparison to the *in vivo* models. While this method may offer good reproducibility, it requires killing of the animals and interpretation of findings may be more difficult compared to the intact animal model. The original method has been subjected to general modification and adapted to several species, including rat, mouse and guinea pig (Skrzypiec-Spring *et al*, 2007). In the Langendorff heart model, the heart is removed from

the anaesthetised animal and the aorta is perfused with an oxygenated medium through both ventricles. A major adaptation of this procedure is represented by the 'working heart' model, in which the left atrium is perfused. The technical details for the preparation and the respective advantages of the two perfusion models are discussed in detail by Sutherland and Hearse (2000) and Mehendale (2001). The isolated heart models exhibit synchronised spontaneous rate under opportune perfusive conditions and is valuable for assessing direct effects of drugs on contractile function, heart rate, coronary vascular tone, cardiac metabolic parameters, and cardiac electrical activity because electrocardiographic as well as monophasic action potential recordings are possible.

Isolated heart preparations based on the Langendorff heart model are used for the screening of anti-arrhythmic and pro-arrhythmic activities of drugs. Hamlin *et al* (2004) tested 26 compounds thought to have pro-arrhythmic activity in humans against 13 other compounds taken as negative controls, in a perfused guinea pig heart preparation. The *ex vivo* study confirmed the pro-arrhythmic effects of the 26 compounds thought to be active in humans and did not show any false positive responses. Eckardt *et al* (1998) used a perfused rabbit heart model to test the pro-arrhythmic potential of 3 anti-arrhythmic class III agents. Finally, approximately 70 clinically used drugs were subjected to blinded evaluation of the pro-arrhythmic potential for the validation of a screening model based on a paced Langendorff perfused rabbit heart model (Hondeghem and Hoffmann, 2003; Hondeghem *et al*, 2003; Valentin *et al*, 2004). Other models, as for instance the arterially perfused rabbit ventricular wedge preparation, are probably as predictive as the Langendorff heart, but technically much more challenging (Lawrence *et al*, 2005; Chen *et al*, 2006). One potential limitation of all of these models is that it is the heart chambers that are perfused, not the coronary arterial circulation. This distinction may lead to an underestimation of the impact of treatment on the performance of the cardiac innervation and musculature.

10.4.2 Purkinje fibres

Beside the isolated perfused heart, isolated Purkinje fibres, most commonly from dogs and rabbits, are often used to evaluate the electrophysiologic effects of cardiac and non-cardiac drugs in terms of prolongation of the action potential duration and induction of early after-depolarisations (early after-depolarisations: Lu *et al*, 2000, 2001a, 2002; Gintant *et al*, 2001; example droperidol: Adamantidis *et al*, 1993, 1994; example sotalol: Wyse *et al*, 1993). Action potential duration prolongation and early after-depolarisations have been proposed as mechanisms responsible for Torsades de Pointes arrhythmias. Although requiring skilled operators, the Purkinje fibre preparation also offers many possibilities for the investigator to easily vary experimental conditions in order to simulate (potentially) pro-arrhythmic risk factors *in vitro*, e.g. female gender, pacing rate, temperature, potassium and/or magnesium concentration in the tissue bath solution, acidosis, and others.

10.5 In vivo models

Finally, one could imagine several *in vivo* models in addition to and beyond the conscious dog to test for cardiac sensitisation potential. For example, the safety pharmacology assessment of the pro-arrhythmic potential of human pharmaceuticals can be performed based on results obtained in anaesthetised dog or pig models, or mostly for screening purposes in telemetry device-implanted conscious guinea pigs and rats. For a detailed overview on the available experimental animals models of cardiac arrhythmias and Torsades de Pointes, the reader is referred to several excellent reviews (e.g. Eckardt *et al*, 1998; Janse *et al*, 1998).

11. CONCLUSIONS AND RECOMMENDATIONS

Cardiac sensitisation is often the primary toxicological endpoint of interest where humans are potentially exposed to high concentrations of halogenated and unsubstituted hydrocarbons for short durations, in fire suppression, propellant and refrigeration applications. The current cardiac sensitisation test is distinct from regulatory guideline toxicity tests, many of which rely on strict application of statistics to aid in interpretation of the results. This document describes the theoretical and practical considerations necessary to properly generate, interpret and apply cardiac sensitisation test data in a manner consistent with scientifically defensible human risk assessments.

Some key considerations related to the cardiac sensitisation test described in the document are provided in the following summary statements.

11.1 The adrenaline-treated dog is the current test system of choice

- There are no clear alternatives to the adrenaline-treated dog.
- The test has evolved and been used successfully for almost 40 years.
- Safe exposure levels established using this test have been demonstrated to be protective of human cardiac sensitisation.
- The test has demonstrated reproducible results.

11.2 The test is highly conservative (protective)

- Administered adrenaline doses produce regional concentrations in large excess of any physiological adrenaline concentrations. Adrenaline doses should be titrated and individualised for each animal (up to 12 µg/kgbw) or a range of adrenaline doses (up to 12 µg/kgbw) should be used at each test gas concentration. The adrenaline should be delivered intravenously at a constant rate over approximately 10 seconds.
- A positive cardiac sensitisation response in a single dog constitutes an effect level. The exposure level at which the positive response occurs is treated as the LOEL. Negative responses in at least 6 dogs are required to assign a NOEL.
- The adrenaline dose challenges the test animals to such an extent that it overwhelms any differential response associated with sex or animal variability.
- Since treatment with adrenaline produces a highly sensitive model, uncertainty or safety factors are not warranted with the described test design, and should not be applied to the dog data used in human risk assessment calculations.

11.3 Interpretation of results should be done by experienced practitioners

- Each animal serves as its own control and a detailed evaluation of ECG data is required.
- Every positive cardiac sensitisation response is potentially fatal. A positive response resulting in death should not be interpreted as more severe than a positive response in which the animal recovers.
- Endpoints other than cardiac sensitisation may not be appropriate for evaluation in animals highly stressed by exogenous adrenaline.
- Cardiac sensitisation mechanisms may be complex and diverse. The precise mechanism involved in cardiac sensitisation for a particular chemical may not be fully known. However, the test results are valid with or without an understanding of the mechanism involved in production of a positive response.

11.4 Cardiac Sensitisation test results can be further interpreted using kinetic models

- A human PBBK model can be used to relate exposure levels in humans to blood levels associated with cardiac sensitisation in experimental studies.
- Blood to air partition coefficients are essential input into PBBK models.
- The human PBBK model is insensitive to xenobiotic metabolism during short-term exposures (approx 5 minutes).
- The human PBBK model has been used to establish safe exposure times for individuals in the general population.

11.5 Alternatives to current test system

- Application of the '3Rs' principle might point to the need to develop an alternative protocol to assess this effect, at least at a screening level.
- One or more alternatives to the current test system may be possible in the future, using either *in vitro* or *in vivo* techniques.
- One potential model might be based on the assessment of cardiac function using telemetry in conscious rats.
- Any viable alternative protocol would require validation against historical cardiac sensitisation data and acceptance by the scientific and regulatory communities.

12. BIBLIOGRAPHY

12.1 References quoted

Abi-Gerges N, Small BG, Lawrence CL, Hammond TG, Valentin JP, Pollard CE. 2004. Evidence for gender differences in electrophysiological properties of canine Purkinje fibers. *Br J Pharmacol* 142:1255-1264.

Abraham MH, Lieb WR, Franks NP. 1991. Role of hydrogen bonding in general anesthesia. *J Pharm Sci* 80:719-724.

Adamantidis MM, Kerram P, Caron JF, Dupuis BA. 1993. Droperidol exerts dual effects on repolarization and induces early afterdepolarizations and triggered activity in rabbit Purkinje fibers. *J Pharmacol Exp Ther* 266:884-893.

Adamantidis MM, Kerram P, Dupuis BA. 1994. *In-vitro* electrophysiological detection of iatrogenic arrhythmogenicity. *Fundam Clin Pharmacol* 8:391-407.

Aguado-Matorras A. 1974. Comparative study of myocardial sensitization to norepinephrine under halothane and enflurane anesthesia. *Acta Anaesthesiol Belgica* 25:198-205.

Alexander DJ, Libretto SE. 1995. An overview of the toxicology of HFA-134a (1,1,1,2-tetrafluoroethane). *Human Exper Toxicol* 14:715-720.

Allied Signal. 1997. Commercial-in-confidence notification of new substances, HFC-245fa, Annex VIIA, Vol 1, Summary of information. Summary of notification dossier of a new chemical substance in accordance with Directive 92/32/EEC (Articles 7/8/9/12), OJ L 154, 35, 5 June 1992. Unpublished report 97-00-0011-11. Allied Signal, Heverlee, Belgium.

Andersen ME, Clewell HJ, Frederick CB. 1995. Contemporary issues in toxicology: applying simulation modeling to problems in toxicology and risk assessment - a short perspective. *Toxicol Appl Pharmacol* 133:181-187.

Anthony BL, Dennison RL, Aronstam RS. 1989. Disruption of muscarinic receptor-G protein coupling is a general property of liquid volatile anesthetics. *Neurosci Lett* 99:191-196.

Antti-Poika M, Heikkila J, Saarinen L. 1990. Cardiac arrhythmias during occupational exposure to fluorinated hydrocarbons. *Br J Ind Med* 47:138-140.

Asahi Glass. 1995a. HCFC-225cb: Assessment of cardiac sensitization potential in dogs. Unpublished report ASG 5/950265. Huntington Research Centre, Huntingdon, Cambridgeshire, England, UK. Asahi Glass, Kanagawa-ku Yokohama, Japan.

Asahi Glass. 1995b. HCFC-225ca/HCFC-225cb (45:55) mixture: Assessment of cardiac sensitization potential in dogs. Unpublished report ASG 6/950266. Huntington Research Centre, Huntingdon, Cambridgeshire, England, UK. Asahi Glass, Kanagawa-ku Yokohama, Japan.

Atlee JL, Malkinson CE. 1982. Potentiation by thiopental of halothane-epinephrine-induced arrhythmias in dogs. *Anesthesiology* 57:285-288.

Azar A, Reinhardt CF, Maxfield ME. 1972. Experimental human exposures to fluorocarbon 12 (dichlorodifluoromethane). *Am Ind Hyg Assoc J* 33:207-216.

Azar A, Trochimowicz HJ, Terrill JB, Mullin LS. 1973. Blood levels of fluorocarbon related to cardiac sensitization. *Am Ind Hyg Assoc J* 34:102-109.

Bamforth BJ, Siebecker KL, Kraemer R. 1961. Effect of epinephrine on: the dog heart during methoxyflurane anesthesia. *Anesthesiology* 22:169-173.

Barbuti A, Ishii S, Shimizu T, Robinson RB, Feinmark SJ. 2002. Block of the background K^+ channel TASK-1 contributes to arrhythmogenic effects of platelet-activating factor. *Am J Physiol* 282:H2024-H2030.

Bass M. 1970. Sudden sniffing deaths. *J Amer Med Assoc* 212:2075-2079.

Beck PS, Clark DG, Tinston DJ. 1973. The pharmacologic actions of bromochlorodifluoro-methan (BCF). *Tox Appl Pharmacol* 24:20-29.

Belji MA, Smith DG, Aviado DM. 1974. Toxicology of aerosol propellant in the respiratory and circulatory system IV: Cardiotoxicity in the monkey. *Toxicology* 2:381-395.

Bell AP. 1951. Death from trichloroethylene in a dry-cleaning establishment. *New Zeal Med J* 50:119-126.

Bidoggia H, Maciel JP, Capalozza N, Mosca S, Blaksley EJ, Valverde E, Bertran G, Arini P, Biagetti MO, Quinteiro RA. 2000. Sex differences on the electrocardiographic pattern of cardiac repolarization: possible role of testosterone. *Am Heart J* 140:678-683.

Böhm M, Schmidt U, Schwinger RHG, Böhm S, Erdmann E. 1993. Effects of halothane on β-adrenoceptors and M-cholinoceptors in human myocardium: radioligand binding and functional studies. *J Cardiovasc Pharmacol* 21:296-304.

Böhm M, Schmidt U, Gierschik P, Schwinger RHG, Böhm S, Erdmann E. 1994. Sensitization of adenylate cyclase by halothane in human myocardium and S49 lymphoma wild-type and *cyc*- cells: evidence for inactivation of the inhibitory G protein $G_{i\alpha}$. *Mol Pharmacol* 45:380-389.

Bosnjak ZJ, Supan FD, Rusch NJ. 1991. The effects of halothane, enflurane, and isoflurane on calcium current in isolated canine ventricular cells. *Anesthesiology* 74:340-345.

Bridle PA, Brosnihan KB, Speth RC, Goormastic M, Ferrario CM. 1983. Basal levels of plasma epinephrine and norepinephrine in the dog. *Hypertension* 5SV:128-33.

Brock WJ, Trochimowicz HJ, Millischer RJ, Farr C, Kawano T, Rusch GM. 1995. Acute and subchronic toxicity of 1,1-dichloro-1-fluoroethane (HCFC-141b). *Food Chem Toxicol* 33:483-90.

Brock WJ, Rusch GM, Trochimowicz HJ. 2003. Cardiac sensitization: methodology and interpretation in risk assessment. *Regul Toxicol Pharmacol* 38:78-90.

Brouillette J, Lupien MA, St-Michel C, Fiset C. 2007. Characterization of ventricular repolarization in male and female guinea-pigs. *J Mol Cell Cardiol* 42:357-366.

Brown BR, Tatum EN, Crout JR. 1972. The effects of inhalation anesthetics on the uptake and metabolism of 1-^{3}H-norepinephrine in guinea-pig atria. *Anesthesiology* 36:263-267.

Burgison RM, O'Malley WE, Heiss CK, Forrest JW, Krantz JC. 1955. Anesthesia XLVI. Fluorinated ethylenes and cardiac arrhythmias induced by epinephrine. *J Pharmacol Exp Ther* 114:470-472.

Calm JM. 1996. The toxicology of refrigerants. In International Refrigeration Conference at Purdue proceedings, 23-26 July 1996. Purdue University, West Lafayette, Indiana, USA.

Cannon WB. 1919. Studies in the conditions of activity in endocrine glands. *Amer J Physiol* 50:399-432.

Carceles MD, Laorden ML, Hernandez J, Miralles FS, Campos M. 1990. Halothane, isoflurane and enflurane potentiate the effect of noradrenaline on ventricular automaticity in the rat heart: evidence of the involvement of both α- or β-adrenoceptors. *J Pharm Pharmacol* 42:186-190.

Carr J, Burgison RM, Vitcha JF. 1949. Anesthesia XXXIV. Clinical constitution of hydrocarbons and cardiac automaticity. *J Pharmacol Exp Ther* 97:1-3.

Chen XD, Yamakage M, Yamada Y, Tohse N, Mamiki A. 2002. Inhibitory effects of volatile anesthetics on currents produced on heterologous expression of KvLQT1 and minK in *Xenopus* oocytes. *Vasc Pharmacol* 39:33-38.

Cheng JH. 2006. Evidences of the gender-related differences in cardiac repolarization and the underlying mechanisms in different animal species and human. *Fundam Clin Pharmacol* 20:1-8.

Chengelis CP. 1993. Acute cardiac sensitization study in dogs with FM-200 by inhalation. Unpublished report WIL-12265. WIL Research Laboratories, Ashland, Ohio, USA.

Chengelis CP. 1995. Current methods for assessing cardiac sensitization potential. Presented at 16th annual meeting of American College of Toxicology, Leesburg Pike, Vienna Virginia, USA, p 34 [Abstract].

Chengelis CP. no date. Cardiac sensitization scoring: Scoring of cardiac arrhythmias for cardiac sensitization. WIL Research Laboratories, Ashland, Ohio, USA.

Chenoweth MB. 1946. Ventricular fibrillation induced by hydrocarbons and epinephrine. *J Industr Hyg Toxicol* 28:151-158.

Chiou JS, Krishna PR, Kamaya H, Ueda I. 1992. Alcohols dehydrate lipid membranes: an infrared study on hydrogen bonding. *Biochim Biophys Acta* 1110:225-233.

Chlopcikova S, Psotova J, Miketova P. 2001. Neonatal cardiomyocytes – a model for the study of morphological, biochemical and electrophysiological characteristics of the heart. *Biomed Papers* 145:49-55.

Chou YH, Wei J, Lin C. 1991. Halothane inhibits residual fast sodium channels in human atrial muscle. *Anesthesiology* 75:716-717.

Clark DG, Tinston DJ. 1973. Correlation of the cardiac sensitising potential of halogenated hydrocarbons with their physicochemical properties. *Brit J Pharmacol* 49:355-357.

Clark DG, Tinston DJ. 1982. Acute inhalation toxicity of some halogenated and non-halogenated hydrocarbons. *Hum Toxicol* 1:239-47.

Clewell, HJ, Andersen ME. 1995. Risk Assessment extrapolation and physiological modeling. *Toxicol Ind Health* 1:111-131.

Correa AM. 1998. Gating kinetics of Shaker K^+ channels are differentially modified by general anesthetics. *Am J Physiol* 275:C1009-C1021.

Davies LA, Hopkins PM, Boyett MR, Harrison SM. 2000. Effects of halothane on the transient outward K^+ current in rat ventricular myocytes. *Br J Pharmacol* 131:223-230.

Detweiler DK. 1981. The use of electrocardiography in toxicological studies with beagle dogs. In T. Balazs T, ed. *Cardiac Toxicology* vol 3. CRC Press, Boca Raton, Florida, USA, pp 33-82.

Drici MD, Clement N. 2001. Is gender a risk factor for adverse drug reactions? The example of drug-induced long QT syndrome. *Drug Safety* 24:575-585.

Du Pont. 1968. Cardiac sensitization screening studies [of several compounds including 1,2 - dichlorohexafluorocyclobutane (fluorocarbon C-316) in dogs]. Unpublished report 93-68. Reinhardt C. Haskell Laboratory for Toxicology and Industrial Medicine, EI Du Pont de Nemours, Newark, Delaware, USA.

Du Pont. 1973. Cardiac sensitization [study of chlorofluoroethane (F-31) in dogs]. Unpublished report 181-73. Mullin LS, Trochimowicz HJ. Haskell Laboratory for Toxicology and Industrial Medicine, EI Du Pont de Nemours, Newark, Delaware, USA.

Du Pont. 1994. HFC-236fa: Assessment of cardiac sensitization potential in dogs. Unpublished report 293-931308. Haskell Laboratory for Toxicology and Industrial Medicine, EI Du Pont de Nemours, Newark, Delaware, USA.

ECETOC. 1990. Dichlorofluoromethane (HCFC-21). Joint Assessment of Commodity Chemicals No. 16. European Centre for Ecotoxicology and Toxicology of Chemicals, Brussels, Belgium.

ECETOC. 2005. 1,1-Dichloro-2,2,2-trifluoroethane (HCFC-123) CAS No. 306-83-2 (third edition). European Centre for Ecotoxicology and Toxicology of Chemicals, Brussels, Belgium.

Eckardt L, Haverkamp W, Borggrefe M, Breithardt G. 1998. Experimental models of Torsades de Pointes. *Cardiovasc Res* 39:178-193.

Edling C, Ohlson CG, Ljungkvist G, Oliv A, Soederholm B. 1990. Cardiac arrhythmia in refrigerator repairmen exposed to fluorocarbons. *Br J Ind Med* 47:207-212.

Egeland GM, Bloom TF, Schnorr TM, Hornung RW, Suruda AJ, Wille KK. 1992. Fluorocarbon 113 exposure and cardiac dysrhythmia among aerospace workers. *Am J Ind Med* 22:851-857.

Eger EI. 1993. A proposal to study the arrhytmogenic capacity of fluorocarbons. Personal communication to Reinhold R, Buffalo Research Laboratories, Allied Signal, Buffalo, New York, USA. Department of Anesthesia, University of California, San Francisco, California, USA. <Eger EI. 1993.pdf>

Eger EI. 1994. Report on arrhytmogenic and other effects of CH_3CFCl_2. Department of Anesthesia, University of California, San Francisco, California, USA. Allied Signal, Buffalo, New York, USA. <Eger EI. 1994.pdf>

Eger EI, Ionescu P, Laster MJ, Gong D, Hudlicky T, Kendig JJ, Harris RA, Trudell JR, Pohorille A. 1999. Minimum alveolar anesthetic concentration of fluorinated alkanols in rats: relevance to theories of narcosis. *Anesth Analg* 88:867-876.

El-Maghrabi EA, Eckenhoff RG, Shuman H. 1992. Saturable binding of halothane to rat brain synaptosomes. *Proc Natl Acad Sci* 89:4329-4332.

Emmen HH, Hoogendijk EMG, Klopping-Ketelaars WAA, Muijser H, Duistermaat E, Ravensberg JC, Alexander DJ, Borkhataria D, Rusch GM, Schmit B. 2000. Human safety and pharmacokinetics of the CFC alternative propellants HFC-134a (1,1,1,2-tetrafluoroethane) and HFC-227 (1,1,1,2,3,3,3-heptafluoropropane) following whole-body exposure. *Regul Toxicol Pharmacol* 32:33-35.

Eskinder H, Rusch NJ, Supan FD, Kampine JP, Bosnjak ZJ. 1991. The effect of volatile anesthetics on L- and T-type calcium channel currents in canine cardiac Purkinje cells. *Anesthesiology* 74:919-926.

Eskinder H, Supan FD, Turner LA, Kampine JP, Bosnjak ZJ. 1993. The effects of halothane and isoflurane on slowly inactivating sodium current in canine cardiac Purkinje cells. *Anesth Analg* 77:32-37.

Esler MD, Alvarenga M, Lambert G, Kaye D, Hastings J, Jennings G, Morris M, Schwarz R, Richards J. 2004. Cardiac sympathetic nerve biology and brain monoamine turnover in panic disorder. *Ann NY Acad Sci* 1018:505-514.

Esler M, Alvarenga M, Pier C, Richards J, El-Osta A, Barton D, Haikerwal D, Kaye D, Schlaich M, Guo L, Jennings G, Socratous F, Lambert G. 2006. The neuronal noradrenaline transporter, anxiety and cardiovascular disease. *J Psychopharmacol* 20S:60-66.

Feil A. 1933. Le benzolisme professional. *Presse Med* 41:129-130.

Foll GE. 1976. Results of toxicology testing on Arctons 123 and 124 at Central Toxicology Laboratory. Personal communication. Attachment to correspondence to Berenbaum MB, Allied Chemical, Morristown, New Jersey, USA. ICI Central Toxicology Laboratory, Macclesfield, Cheshire, UK [Cited by ECETOC, 2005].

Franks NP, Lieb WR. 1984. Do general anaesthetics act by competitive binding to specific receptors? *Nature* 310:599-601.

Franks NP, Lieb WR. 1993. Selective actions of volatile general anaesthetics at molecular and cellular levels. *Br J Anaesthesia* 71:65-76.

Franks NP, Lieb WR. 1994. Molecular and cellular mechanisms of general anaesthesia. *Nature* 367:607-614.

Freeman LC, Muir WW. 1991a. Effects of halothane on impulse propagation in Purkinje fibers and at Purkinje-muscle junctions: relationship of V_{max} to conduction velocity. *Anesth Analg* 72:5-10.

Freeman LC, Muir WW. 1991b. α-Adrenoceptor stimulation in the presence of halothane: effects on impulse propagation in cardiac Purkinje fibers. *Anesth Analg* 72:11-17.

Fu J, Gao J, Pi R, Liu P. 2005. Na optimized protocol for culture of cardiomyocyte from neonatal rat. *Cytotechnology* 49:109-116.

Fujikawa H, Sato Y, Arakawa H, Mitsuhashi T, Minezaki K, Kuroki S, Sekiguchi H, Nakayama T, Ikeda U, Shimada K. 1998. Induction of Torsades de Pointes by dobutamine infusion in a patient with idiopathic long QT syndrome. *Int Med* 37:149-152.

Fujita H, Ogura T, Tamagawa M, Uemura H, Sato T, Ishida A, Imamaki M, Kimura F, Miyazaki M, Nakaya H. 2006. A key role for the subunit SUR2B in the preferential activation of vascular K_{ATP} channels by isoflurane. *Br J Pharmacol* 149:573-580.

Fülöp L, Banyasz T, Szabo G, Toth IB, Biro T, Lorincz I, Balogh A, Peto K, Miko I, Nanasi PP. 2006. Effects of sex hormones on ECG parameters and expression of cardiac ion channels in dogs. *Acta Physiol* 188:163-171.

Furukawa T, Kurokawa J. 2007. Regulation of ion channels via non-genomic action of sex steroid hormones: implication for the gender difference in cardiac arrhythmias. *Pharmacol Ther* 115:106-115.

Gallagher JD, Weindling SN, Anderson G, Fillinger MP. 1998. Effects of sevoflurane on QT interval in a patient with congenital long QT syndrome. *Anesthesiology* 89:1569-1573.

Garb S, Chenoweth MB. 1948. Studies on hydrocarbon-epinephrine induced ventricular fibrillation. *J Pharmacol Exp Ther* 94:12-18.

Gintant GA, Limberis JT, McDermott JS, Wegner CD, Cox BF. 2001. The canine Purkinje fiber: an *in vitro* model system for acquired long QT syndrome and drig-induced arrhythmogenesis. *J Cardiovasc Pharmacol* 37:607-618.

Grant AO, Tranquillo J. 2007. Action potential and QT prolongation not sufficient to cause Torsades de Pointes: role of action potential triangulation. *J Cardiovasc Electrophysiol* 18:204-205.

Guo D, Zhao XJ, Wu Y, Liu TX, Kowey PR, Yan GX. 2007. L-type calcium current reactivation contributes to arrhythmogenesis associated with action potential triangulation. *J Cardiovasc Electrophysiol* 18:196-203.

Hamilton A. 1925. Industrial poisons in the United States. Macmillan, New York, USA, pp 457-461.

Hamlin RL, Cruze CA, Mittelstadt SW, Kijtawornrat A, Keene BW, Roche BM, Nakayama T, Nakayama H, Hamlin DM, Arnold T. 2004. Sensitivity and specificity of isolated perfused guinea-pig heart to test for drug-induced lengthening of QTc. *J Pharmacol Toxicol Methods* 49:15-23.

Harary I, Farley B. 1963. *In vitro* studies on single beating rat heart cells II: intercellular communication. *Exp Cell Res* 29:466-474.

Hardy CJ, Sharman IJ, Clark GC. 1991. Assessment of cardiac sensitisation in dogs: Comparison of HFA134a and A12. Unpublished report CTL/C/2521. ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. <JACC50>

Hardy CJ, Kieran PC. 1992. Hydrofluorocarbon 32: Assessment of cardiac sensitisation potential in Beagle dogs. Unpublished report ISN 276/920493. Huntington Research Centre, Huntingdon, Cambridgeshire, England, UK. Programme for Alternative Fluorocarbon Toxicity Testing, RAND Environmental Science and Policy Center, Arlington, Virginia, USA.

Hardy CJ, Kieran PC, Sharman IJ. 1994. Assessment of the cardiac sensitisation potential of a range of halogenated alkanes. *Toxicologist* 14:378 [Abstract].

Hashimoto K, Hashimoto K. 1972. The mechanism of sensitization of the ventricle to epinephrine by halothane. *Am Heart J* 83:652-658.

Hermann H, Vial J. 1934. Syncope adrenalino-mono-chloroethanique. *C R Soc Biol* 117:439-440.

Hermann H, Vial J. 1935. Nouvelles syncopes cardiaques par association toxique de l'adrénaline et de divers produits organiques volatils. *C R Soc Biol* 119:1316-1317.

Hescheler J, Halbach M, Egert U, Lu ZJ, Bohlen H, Fleischmann BK, Reppel M. 2004. Determination of electrical properties of ES cell-derived cardiomyocytes using MEAs. *J Electrocardiol* 37S:110-116.

Himmel. 2008. Mechanisms involved in cardiac sensitization by volatile anesthetics: General applicability to halogenated hydrocarbons? *Crit Rev Toxicol* 38:773-803. <ECETOC>

Hirota K, Ito Y, Masuda A, Momose Y. 1989. Effects of halothane on membrane ionic currents in guinea-pig atrial and ventricular myocytes. *Acta Anaesthesiol Scand* 33:239-244.

Hirota K, Ito Y, Kuze S, Momose Y. 1992. Effects of halothane on electrophysiologic properties and cyclic adenosine 3',5'-monophosphate content in isolated guinea-pig hearts. *Anesth Analg* 74:564-569.

Hondeghem LM, Lu HR, van Rossem K, De Clerck F. 2003. Detection of proarrhythmia in the female rabbit heart: blinded validation. *J Cardiovasc Electrophysiol* 14:287-294.

Hondeghem LM. 2007. Relative contributions of TRIaD and QT to proarrhythmia. *J Cardiovasc Electrophysiol* 18:655-657.

Hoschek R. 1962. Cases of delayed sudden death after slight exposure to trichloroethylene. *Int Arch Gewerbepath* 19:319-328.

Hüneke R, Fassl J, Rossaint R, Lückhof A. 2004. Effects of volatile anesthetics on cardiac ion channels. *Acta Anaesthesiol Scand* 48:547-561.

Hussain M, Leach A, Hardy CJ. 2006. A refined method of restraint for dogs used in inhalation studies. *Toxicologist* 90S1: [Abstract 1900].

Ikemoto Y, Yatani A, Imoto Y, Arimura H. 1986. Reduction in the myocardial sodium current by halothane and thiamylal. *Jap J Physiol* 36:107-121.

James AF, Arberry LA, Hancox JC. 2004. Gender-related differences in ventricular myocyte repolarization in the guinea-pig. *Basic Res Cardiol* 99:183-192.

Janse MJ, Opthof T, Kleber AG. 1998. Animal models of cardiac arrhythmias. *Cardiovasc Res* 39:165-177.

Jiao Z, de Jesus VR, Iravanian S, Campbell DP, Xu J, Vitali JA, Banach K, Fahrenbach J, Dudley SC. 2006. A possible mechanism of halocarbon-induced cardiac sensitization arrhythmias. *J Mol Cell Cardiol* 41:698-705.

Johnston RR, Eger EI, Wilson C. 1976. A comparative interaction of epinephrine with enflurane, isoflurane, and halothane in man. *Anesth Analg* 55:709-712.

Kang J, Reynolds WP, Chen XL, Ji J, Wang H, Rampe DE. 2006. Mechanisms underlying the QT interval-prolonging effects of sevoflurane and its interactions with other QT-prolonging drugs. *Anesthesiology* 104:1015-1022.

Kaufman JD, Silverstein MA, Moure-Eraso R. 1994. Atrial fibrillation and sudden death related to occupational solvent exposure. *Amer J Industr Med* 25:731-735.

Kawachi I, Colditz GA, Ascherio A, Rimm EB, Giovannucci E, Stampfer MJ, Willett WC. 1994a. Prospective study of phobic anxiety and risk of coronary heart disease in men. *Circulation* 89:1992-1997.

Kawachi I, Sparrow D, Vokonas PS, Weiss ST. 1994b. Symptoms of anxiety and risk of coronary heart disease. The normative aging study. *Circulation* 90:2225-2229.

Kawano T, Trochimowicz HJ, Malinverno G, Rusch GM. 1995. Toxicological evaluation of 1,1,1,2,2-pentafluoroethane (HFC-125). *Fundam Appl Toxicol* 28:223-231.

Kehoe RA. 1943. Report on human exposure to dichlorodifluoromethane in air. Unpublished report. Kettering Laboratory, University of Cincinnati, Cincinnati, Ohio, USA.

Kenny TJ. 1994. HFCC-1717. Assessement of cardiac sensitisation potential in dogs. Unpublished report ALS 57/942811. Huntingdon Research Centre, Huntingdon, Cambrigeshire, England UK. Allied-Signal, Morristown, New Jersey, USA.

Kleinfeld M, Tabershaw IR. 1954. Trichloroethylene toxicity. *Arch Industr Hyg Occup Med* 10:134-141.

Krantz JC Jr, Carr CJ, Vitcha JF. 1948. Anesthesia XXXI. A study of cyclic and noncyclic hydrocarbons on cardiac automaticity. *J Pharmacol Exp Ther* 94:315-318.

Krasowski MD, Harrison NL. 1999. General anaesthetic actions on ligand-gated ion channels. *Cell Mol Life Sci* 55:1278-1303.

Kuenszberg E, Loeckinger A, Kleinsasser A, Lindner KH, Puehringer F, Hoermann C. 2000. Sevoflurane progressively prolongs the QT interval in unpremedicated female adults. *Eur J Anaesthesiol* 17:662-664.

Kulier AH, Turner LA, Vodanovic S, Contney S, Lathrop DA, Bosnjak ZJ. 2000. Multiple agents potentiate α_1-adrenoceptor-induced conduction depression in canine cardiac Purkinje fibers. *Anesthesiology* 92:1713-1721.

Langendorff O. 1895. Untersuchungen am überlebenden Saügertierherzen. *Pflugers Arch ges Physiol* 61:291-332.

Laster MJ, Johnson BH, Eger EI, Taheri S. 1990. A method for testing for epinephrine-induced arrhythmias in rats. *Anesth Analg* 70:654-657.

Lawrence CL, Pollard CE, Hammond TG, Valentin JP. 2005. Nonclinical proarrhythmia models: predicting Torsades de Pointes. *J Pharmacol Toxicol Methods* 52:46-59.

Levy AG, Lewis T. 1911-1912. Heart irregularities, resulting from the inhalation of low percentages of chloroform vapour, and their relationship to ventricular fibrillation. *Heart* 3:99-111.

Levy AG. 1913. The exciting causes of ventricular fibrillation in animals under chloroform anesthesia. *Heart* 4:319-378.

Liu XK, Katchman A, Drici MD, Ebert SN, Ducic I, Morad M, Woosley RL. 1998. Gender difference in the cycle length-dependent QT and potassium currents in rabbits. *J Pharmacol Exp Ther* 285:672-679.

Liu XK, Katchman A, Whitfield BH, Wan G, Janowski EM, Woosley RL, Ebert SN. 2003. *In-vivo* androgen treatment shortens the QT interval and increases the densities of inward and delayed rectifier potassium currents in orchiectomized male rabbits. *Cardiovasc Res* 57:28-36.

Locati EH, Zareba W, Moss AJ, Schwartz PJ, Vincent M, Lehmann MH, Towbin JA, Priori SG, Napolitano C, Robinson JL, Andrews M, Timothy K, Hall WJ. 1998. Age- and sex-related differences in clinical manifestations in patients with congenital long-QT syndrome. Findings from the international LQTS registry. *Circulation* 97:2237-2244.

Lown B, Wolf M. 1971. Approaches to sudden death from coronary heart disease. *Circulation* 44:130-142.

Lu HR, Marien R, Saels A, de Clerck F. 2000. Are there sex-specific differences in ventricular repolarization or in drug-induced early afterdepolarizations in isolated rabbit Purkinje fibers? *J Cardiovasc Pharmacol* 36:132-139.

Lu HR, Marien R, Saels A, de Clerck F. 2001a. Species plays an important role in drug-induced prolongation of action potential duration and early after depolarizations in isolated Purkinje fibers. *J Cardiovasc Electrophysiol* 12:93-102.

Lu HR, Remeysen P, Somers K, Saels A, de Clerck F. 2001b. Female gender is a risk factor for drug-induced long QT and cardiac arrhythmias in an *in vivo* rabbit model. *J Cardiovasc Electrophysiol* 12:538-545.

Lu HR, Vlaminckx E, van Ammel K, de Clerck F. 2002. Drug-induced long QT in isolated rabbit Purkinje fibers: importance of action potential duration, triangulation and early afterdepolarizations. *Eur J Pharmacol* 452:183-192.

Makkar RR, Fromm BS, Steinman RT, Meissner MD, Lehmann MH. 1993. Female gender as a risk factor for Torsades de Pointes associated with cardiovascular drugs. *JAMA* 270:2590-2597.

Maze M, Smith CM. 1983. Identification of receptor mechanism mediating epinephrine-induced arrhythmias during halothane anesthesia in the dog. *Anesthesiology* 59:322-326.

McCord CP. 1932. Benzol (benzene) poisoning. Industrial Health Conservancy Laboratory, Cincinnati, Ohio, USA.

Meecham KG, Beard DJ, Moore SA, Davies PH, Hardy CJ. 2006. Effects of inhalation dosing procedures on baseline cardiovascular parameters andresponse to D, L-sotalol in conscious telemetered dogs. *Toxicologist* 90S1: [Abstract 657].

Meek WJ, Hathaway HR, Orth OS. 1937. The effects of ether, chloroform and cyclopropane on cardiac automaticity. *J Pharmacol Exp Ther* 61:240-252.

Mehendale HM. 2001. Application of isolated organ perfusion techniques in toxicology. In Wallace Hayes A, ed. *Principle and Methods of Toxicology*. Taylor & Francis, Philadelphia, PA, USA.

Mertens J, Naas D, Severs L, Chengelis C, Biesemeier J. 1995. Cardiac sensitizing properties of 1,1,1,2,3,3,3-heptafluoropropane (HFC-227ea, FM-200). Poster presented at 16th annual meeting of the College of Toxicology, Leesburg Pike, Vienna Virginia, USA, p 40 [Abstract].

Metz S, Maze M. 1985. Halothane concentration does not alter the threshold for epinephrine-induced arrhythmias in dogs. *Anesthesiology* 62:470-474.

Meyer T, Boven KH, Günther E, Fejtl M. 2004. Micro-electrode arrays in cardiac safety pharmacology. A novel tool to study QT interval prolongation. *Drug Safety* 27:763-772.

Milberg P, Hilker E, Ramtin S, Cakir Y, Stypmann J, Engelen MA, Mönnig G, Osada N, Breithardt G, Haverkamp W, Eckardt L. 2007. Proarrhythmia as a class effect of quinolones: increased dispersion of repolarization and triangulation of action potential predict Torsades de Pointes. *J Cardiovasc Electrophysiol* 18:647-654.

Miletich DJ, Khan A, Albrecht RF, Jozefiak A. 1983. Use of heart cell cultures as a tool for the evaluation of halothane arrhythmia. *Toxicol Appl Pharmacol* 70:181-187.

Moore MA, Weiskopf RB, Eger EI, Wilson C. 1993. Arrhythmogenic doses of epinephrine are similar during desflurane or isoflurane anesthesia in humans. *Anesthesiology* 79:943-947.

Morris LE, Noltensmeyer MH, White JM. 1953. Epinephrine induced cardiac irregularities in the dog during anesthesia with trichloroethylene, cyclopropane, ethyl chloride and chloroform. *Anesthesiology* 14:153-158.

Mullin L. 1969. Cardiac sensitisation of 1,1-dichloro-1-fluoroethane (FC-141b). Unpublished report HLR 325-69. Haskell Laboratory for Toxicology and Industrial Medicine, EI Du Pont de Nemours, Newark, Delaware, USA.

Mullin LS, Azar A, Reinhardt CF, Smith PE, Fabryka EF. 1972. Halogenated hydrocarbon-induced cardiac arrhythmias associated with release of endogenous epinephrine. *Am Ind Hyg Assoc J* 33:389-396.

Mullin LS. 1976a. Cardiac sensitization [study in beagle dogs on 1-chloro-1,2,2,2-tetrafluoroethane]. Unpublished report HLR 220-76. Haskell Laboratory for Toxicology and Industrial Medicine. Du Pont de Nemours, Newark, Delaware, USA.

Mullin LS. 1976b. Cardiac sensitization [study in beagle dogs on 1,2-dichloro-1,1-difluoroethane]. Unpublished report HLR 113-76. Haskell Laboratory for Toxicology and Industrial Medicine. Du Pont de Nemours, Newark, Delaware, USA.

Mullin L. 1977. Cardiac sensitisation of 1,1-dichloro-1-fluoroethane (FC-141b). Unpublished report HLR 957-77. Haskell Laboratory for Toxicology and Industrial Medicine, EI Du Pont de Nemours, Newark, Delaware, USA.

Mullin LS. 1979. FC 134a: Cardiac sensitisation. Unpublished report 42-79. Haskell Laboratory for Toxicology and Industrial Medicine. Du Pont de Nemours, Newark, DE, USA.

Mullin LS, Reinhardt CF, Hemingway RE. 1979. Cardiac arrhythmias and blood levels associated with inhalation of Halon 1301. *Am Ind Hyg Assoc J* 40:653-658.

Nakao S, Hirata H, Kagawa Y. 1989. Effects of volatile anesthetics on cardiac calcium channels. *Acta Anaesthesiol Scand* 33:326-330.

National Safety Council. 1926. Final report of the Committee on Benzol, National Safety Council, Chemical and Rubber Sections. National Bureau of Casualty and Surety Underwriters, New York, USA, pp 10-18.

Navarro R, Weiskopf RB, Moore MA, Lockhart S, Eger EI, Koblin D, Lu G, Wilson C. 1994. Humans anesthetized with sevoflurane or isoflurane have similar arrhythmic response to epinephrine. *Anesthesiology* 80:545-549.

NFPA. 2004. Guidance for clean fire suppressant agents 2001. National Fire Protection Association, Quincy, Massachusetts, USA.

Niggli E, Rüdisüli A, Maurer P, Weingart R. 1989. Effects of general anesthetics on current flow across membranes in guinea-pig myocytes. *Am J Physiol* 256:C273-C281.

NIOSH Alert. 1989. Request for assistance in preventing death from excessive exposure to chlorofluorocarbon 113 (CFC-113). DHHS (NIOSH) Publication No. 89-109. Department of Health and Human Services, Washington DC, USA [http://www.cdc.gov/niosh/89-109.html].

Offenhäusser A, Knoll W. 2001. Cell-transistor hybrid systems and their potential applications. Trends in Biotechnol. 19, 2: 62-66.

Pancrazio JJ. 1996. Halothane and isoflurane preferentially depress a slowly inactivating component of Ca^{2+} channel current in guinea-pig myocytes. *J Physiol* 494:91-103.

Pantaleoni GC, Luzi V. 1975a. Cardiotoxicity of monochlorodifluoroethane (contribution to the study on the toxicopathology of sniffing syndrome). Note 3. *Rass Med Sper* **22:**255-269.

Pantaleoni GC, Luzi V. 1975b. Cardiotoxicity of monochlorodifluoroethane (contribution to the study on the toxicopathology of sniffing syndrome). Note 4. *Rass Med Sper* **22:**270-274.

Patel AJ, Honore E, Lesage F, Fink M, Romey G, Lazdunski M. 1999. Inhalational anesthetics activate two-pore-domain background K^+ channels. *Nature Neurosci* 2:422-426.

Patel MK, Mistry D, John III JE, Mounsey JP. 2000. Sodium channel isoform-specific effects of halothane: protein kinase C coexpression and slow inactivation gating. *Br J Pharmacol* 130:1785-1792.

Pham TV, Rosen MR. 2002. Sex, hormones, and repolarization. *Cardiovasc Res* 53:740-751.

Philips FS, Gilman A, Crescitelli FN. 1946. Studies on the pharmacology of DDT (2,2,*bis-para*-chlorophenyl-1,1,1-trichloroethane) II. The sensitization of the myocardium to sympathetic stimulation during acute DDT intoxication. *J Pharmacol Exp Ther* 86:222-228.

Pillekamp F, Reppel M, Brockmeier K, Hescheler J. 2006. Impulse propagation in late-stage embryonic and neonatal murine ventricular slices. *J Electrocardiol* 39:425.e1-425.e4.

Price HL, Lurie AA, Jones RE, Price ML, Linde HW. 1958. Cyclopropane anesthesia II. Epinephrine in initiation of ventricular arrhythmias by carbon dioxide inhalation. *Anesthesiology* 19:619-630.

Puttick RM, Terrar DA. 1992. Effects of propofol and enflurane on action potentials, membrane currents and contration of guinea-pig isolated ventricular myocytes. *Br J Pharmacol* 107:559-565.

Raventos J. 1956. The action of fluothane: A new volatile anesthetic. *Brit J Pharmacol* 11:394-410.

Reinhardt CF, Azar A, Maxfield ME, Smith PE, Mullin LS. 1971. Cardiac arrhythmias and aerosol 'sniffing'. *Arch Environ Hlth* 22:265-279.

Reinhardt CF, Mullin LS, Maxfield ME. 1973. Epinephrine-induced cardiac arrhythmia potential of some common industrial solvents. *J Occup Med* 15:953-955.

Reppel M, Igelmund P, Egert U, Juchelka F, Hescheler J, Drobschinskaya I. 2007. Effect of cardioactive drugs on action potential generation and propagation in embryonic stem cell-derived cardiomyocytes. *Cell Physiol Biochem* 19:213-224.

Reynolds AK. 1984. On the mechanism of myocardial sensitization to catecholamines by hydrocarbon anesthetics. *Can J Physiol Pharmacol* 62:183-198.

Rithalia A, Hopkins PM, Harrison SM. 2004. The effects of halothane, isoflurane, and sevoflurane on Ca^{2+} current and transient outward K^{+} current in subendocardial and subepicardial myocytes from the rat left ventricle. *Anesth Analg* 99:1615-1622.

Roden DM, Balser JR, George AL, Anderson ME. 2002. Cardiac ion channels. *Annu Rev Physiol* 64:431-475.

Rodstein M, Wolloch L, Gubner RS. 1971. Mortality study of the significance of extrasystoles in an insured population. *Circulation* 44:617-625.

Rusch GM, Coombs D, Hardy C. 1999. The acute, genetic, developmental and inhalation toxicology of 1,1,1,3,3-pentafluoropropane (HFC-245fa). *Toxicol Sci* 52:289-301. <JACC44>

Schmeling WT, Warltier DC, McDonald DJ, Madsen KE, Atlee JL, Kampine JP. 1991. Prolongation of the QT interval by enflurane, isoflurane, and halothane in humans. *Anesth Analg* 72:137-144.

Schollmeyer W. 1960. Sudden death due to trichloroethylene poisoning after prolonged exposure to the poison. *Arch Toxik* 18:220-235.

Scholz EP, Alter M, Zitron E, Kiesecker C, Kathöfer S, Thomas D, Kreye VAW, Kreuzer J, Becker R, Katus HA, Greten J, Karle CA. 2006. *In-vitro* modulation of hERG channels by organochlorine solvent trichloromethane as potential explanation for proarrhythmic effects of chloroform. *Toxicol Lett* 165:156-166.

Schulze-Bahr E, Kirchhof P, Eckardt L, Bertrand J, Breithardt G. 2005. Gender differences in cardiac arrhythmias. *Herz* 30:390-400.

Shibata S, Ono K, Iijima T. 2004. Sevoflurane inhibition of the slowly activating delayed rectifier K^+ current in guinea-pig ventricular cells. *J Pharmacol Sci* 95:363-373.

Skaggs SR, Moore TA, Tapscott RE. 1995. Toxicological properties of halon substitutes. In Miziolek AJ, Tsang W, eds. Halon properties, ACS Symposium Series 611 Chapter 10. American Chemical Society, Washington, DC, USA, pp 99-109.

Skrzypiec-Spring M, Grotthus B, Szelag A, Schulz R. 2007. Isolated heart perfusion according to Langendorff – still viable in the new millenium. *J Pharmacol Toxicol Methods* 55:113-126.

Smith RD, Pettway CE. 1975. Absence of sensitization to epinephrine-induced cardiac arrhythmia and fibrillation in dogs and cats anesthetized with CI-744. *Am J Vet Res* 36:695-698.

Stadnicka A, Kwok WM, Hartmann HA, Bosnjak ZJ. 1999. Effects of halothane and isoflurane on fast and slow inactivation of human heart hH1a sodium channels. *Anesthesiology* 90:1671-1683.

Stadnicka A, Bosnjak ZJ, Kampine JP, Kwok WM. 2000. Modulation of cardiac inward rectifier K^+ current by halothane and isoflurane. *Anesth Analg* 90:824-833.

Starling EH, Evans L. 1962. Principles of human physiology, 13th ed. In H Davson, Eggleton MG, eds. *Toxicological Sciences*. Lee and Febiger, Philadelphia, Pennsylvania, USA, p 1413.

Steidl EM, Neveu E, Bertrand D, Buisson B. 2006. The adult rat hippocampal slice revisited with multi-electrode arrays. *Brain Res* 1096:70-84.

Steinbeck G. 1983. Tachykarde Rhythmusstörungen. In Lüderitz B, ed. *Herzrhythmus-störungen*. Springer, Berlin, Germany, pp 617-641.

Stett A, Egert U, Guenther E, Hofmann F, Meyer T, Nisch W, Haemmerle H. 2003. Biological application of microelectrode arrays in drug discovery and basic research. *Anal Bioanal Chem* 377:486-495.

Sumikawa K, Ishizaka N, Suzaki M. 1983. Arrhythmogenic plasma levels of epinephrine during halothane, enflurane, and pentobarbital anesthesia in the dog. *Anesthesiology* 58:322-325.

Sutherland FJ, Hearse DJ. 2000. The isolated blood and perfusion fluid perfused heart. *Pharmacol Res* 41:613-627.

Suzuki A, Bosnjak ZJ, Kwok WM. 2003. The effects of isoflurane on the cardiac slowly activating delayed-rectifier potassium channel in guinea-pig ventricular myocytes. *Anesth Analg* 96:1308-1315.

Takahara A, Sugiyama A, Hashimoto K. 2005a. Reduction of repolarization reserve by halothane anaesthesia sensitizes the guinea-pig heart for drug-induced QT interval prolongation. *Br J Pharmacol* 146:561-567.

Takahara A, Sugiyama A, Satoh Y, Wang K, Honsho S, Hashimoto K. 2005b. Halothane sensitizes the canine heart to pharmacological I_{Kr} blockade. *Eur J Pharmacol* 507:169-177.

Takahashi H, Puttick RM, Terrar DA. 1994. The effects of propofol and enflurane on single calcium channel currents of guinea-pig isolated ventricular myocytes. *Br J Pharmacol* 111:1147-1153.

Tas PWL, Kress HG, Koschel K. 1987. Volatile anesthetics and n-alkanols inhibit the uptake of nor-adrenaline into pheochromocytoma cells. *Anaesthesist* 36:340-344.

Terrar DA, Victory JG. 1988. Influence of halothane on electrical coupling in cell pairs isolated from guinea-pig ventricle. *Br J Pharmacol* 94:509-514.

Terrenoire C, Piriou V, Bonvallet R, Chouabe C, Espinosa L, Rougier O, Tourneur Y. 2000. Opposite effects of halothane on guinea-pig ventricular action potential duration. *Eur J Pharmacol* 390:95-101.

Terrenoire C, Lauritzen I, Lesage F, Romey G, Lazdunski M. 2001. A TREK-1-like potassium channel in atrial cells inhibited by β-adrenergic stimulation and activated by volatile anesthetics. *Circ Res* 89:336-342.

Thompson EB, Galysh FT. 1973. Quantitative assessment of anesthetic-induced cardiac sensitization to epinephrine. *Anesth Analg* 52:800-806.

Tranquilli WJ, Thurmon JC, Benson GJ, Davis LE. 1986. Alteration in the arrhythmogenic dose of epinephrine (ADE) following xylazine administration to halothane-anesthetized dogs. *J Vet Pharmacol Ther* 9:198-203.

Trochimowicz HJ, Mullin LS. 1973. Cardiac sensitisation of fluorocarbon 123. Unpublished report HLR 132 73. Haskell Laboratory for Toxicology and Industrial Medicine. Du Pont de Nemours, Newark, Delaware, USA.

Trochimowicz HJ, Azar A, Terrill JB, Mullin LS. 1974. Blood levels of fluorocarbon related to cardiac sensitization: II. *Am Ind Hyg Assoc J* 35:632-639.

Trochimowicz HJ, Reinhardt CF, Mullin LS, Azar A, Karrh BW. 1976. The effect of myocardial infarction on the cardiac sensitization potential of certain halocarbons. *J Occup Med* 18:26-30.

Turner LA, Marijic J, Kampine JP, Bosnjak ZJ. 1990. A comparison of the effects of halothane and tetrodotoxin on the regional repolarization characteristics of canine Purkinje fibers. *Anesthesiology* 73:1158-1168.

Turner LA, Polic S, Hoffmann RG, Kampine JP, Bosnjak ZJ. 1993a. Actions of halothane and isoflurane on Purkinje fibers in the infarcted canine heart: conduction, regional refractoriness, and reentry. *Anesth Analg* 76:718-725.

Turner LA, Polic S, Hoffmann RG, Kampine JP, Bosnjak ZJ. 1993b. Actions of volatile anesthetics on ischemic and nonischemic Purkinje fibers in the infarcted canine heart: regional action potential characteristics. *Anesth Analg* 76:726-733.

Ueno S, Trudell JR, Eger EI, Harris RA. 1999. Actions of fluorinated alkanols on $GABA_A$ receptors: relevance to theories of narcosis. *Anesth Analg* 88:877-883.

Valentin JP, Hoffmann P, De Clerck F, Hammond TG, Hondeghem L. 2004. Review of the predictive value of the Langendorff heart model (Screenit system) in assessing the proarrhythmic potential of drugs. *J Pharmacol Toxicol Methods* 49:171-181.

Venugopalan CS, Holmes E, O'Malley NA. 1989. Comparison of arrhythmogenic doses of epinephrine in heartworm-infected and noninfected dogs. *Am J Vet Res* 50:1872-1876.

Vinegar A, Jepson GW, Overton JH. 1998. PBPK modeling of short-term (0-5 minutes) human inhalation exposures to halogenated hydrocarbons. *Inhal Toxicol* 10:411-429.

Vinegar A, Jepson GW, Hammann SJ, Harper G, Dierdorf DS, Overton JH. 1999. Simulated blood levels of CF3I in personnel exposed during its release from a F-15 jet engine nacelle and during intentional inhalation. *AIHA Journal* 80:403-408.

Vinegar A, Jepson GW, Cisneros M, Rubenstein R, Brock WJ. 2000. Setting safe exposure levels for Halon replacement chemicals using physiologically-based pharmokinetic modeling. *Inhal Toxicol* 12:751-763.

Vodanovic S, Turner LA, Hoffmann RG, Kampine JP, Bosnjak ZJ. 1997. Actions of phenylephrine, isoproterenol, and epinephrine with halothane on endocardial conduction and activation in canine left ventricular papillary muscles. *Anesthesiology* 87:117-126.

Vos MA, van Opstal JM, Leunissen JDM, Verduyn SC. 2001. Electrophysiologic parameters and predisposing factors in the generation of drug-induced Torsades de Pointes arrhythmias. *Pharmacol Ther* 92:109-122.

Waters RM, Orth OS, Gillespie NA. 1943. Trichloroethylene anesthesia and cardiac rhythm. *Anesthesiology* 4:1-5.

Weigl LG, Schreibmayer W. 2001. G protein-gated inwardly rectifying potassium channels are targets for volatile anesthetics. *Mol Pharmacol* 60:282-289.

Weigt HU, Rehmert GC, Bosnijak ZJ, Kwok WM. 1997. Conformational state-dependent effects of halothane on cardiac Na^+ current. *Anesthesiology* 87:1494-1506.

Weigt HU, Kwok WM, Rehmert GC, Turner LA, Bosnjak ZJ. 1997a. Voltage-dependent effects of volatile anesthetics on cardiac sodium current. *Anesth Analg* 84:285-293.

Weigt HU, Kwok WM, Rehmert GC, Turner LA, Bosnjak ZJ. 1997b. Modulation of cardiac sodium current by α_1-stimulation and volatile anesthetics. *Anesthesiology* 87:1507-1516.

Weigt HU, Kwok WM, Rehmert GC, Bosnjak ZJ. 1998a. Modulation of the cardiac sodium current by inhalational anesthetics in the absence and presence of β-stimulation. *Anesthesiology* 88:114-124.

Weigt HU, Kwok WM, Rehmert GC, Bosnjak ZJ. 1998b. Sensitization of the cardiac Na channel to α_1-adrenergic stimulation by inhalation anesthetics. Evidence for distinct modulatory pathways. *Anesthesiology* 88:125-133.

Weissenburger J, Nesterenko VV, Antzelevitch C. 2000. Transmural heterogeneity of ventricular repolarization under baseline and long QT conditions in the canine heart *in vivo*: Torsades de Pointes develops with halothane but not pentobarbital anesthesia. *J Cardiovasc Electrophysiol* 11:290-304.

Wilkinson DJC, Thompson JM, Lambert GW, Jennings GL, Schwarz RG, Jefferys D, Turner AG, Esler MD. 1998. Sympathetic activity in patients with panic disorder at rest, under laboratory mental stress, and during panic attacks. *Arch Gen Psychiatry* 55:511-520.

Wyse KR, Ye V, Campbell TJ. 1993. Action potential prolongation exhibits simple dose-dependence for sotalol, but reverse dose-dependence for quinidine and disopyramide: implications for proarrhythmia due to triggered activity. *J Cardiovasc Pharmacol* 21:316-322.

Xiang GX, Pan LB, Huang L, Yu ZY, Song XD, Cheng J, Xing W, Zhou YX. 2007. Microelectrode array-based system for neuropharmacological applications with cortical neurons cultured *in vitro*. *Biosensors Bioelectronics* 22:2478-2484.

Yamada M, Hatakeyama N, Malykhina AP, Yamazaki M, Momose Y, Akbarali HI. 2006. The effects of sevoflurane and propofol on QT interval and heterologously expressed human ether-a-go-go-related gene currents in *Xenopus* oocytes. *Anesth Anal* 102:98-103.

Yamamoto K, Tamura T, Imai R, Yamamoto M. 2001. Acute canine model for drug-induced Torsades de Pointes in drug safety evaluation – Influences of anesthesia and validation with quinidine and astemizole. *Toxicol Sci* 60:165-176.

Zordoky BNM, El-Kadi AOS. 2007. H9c2 cell line is a valuable *in vitro* model to study the drug metabolizing enzymes in the heart. *J Pharmacol Toxicol Methods* 56:317-322 [doi:10.1016/j.vascn.2007.06.001].

12.2 References not quoted

The following reference was consulted by the Task Force, but not quoted for the reason given.

*Dorne JLCM. 2007. Review. Human variability in hepatic and renal elimination: implications for risk assessment. *J Appl Toxicol* 27:411-420 [Review].

LIST OF SPECIAL ABBREVIATIONS

≈	Approximately
↓	Decrease, reduction
↑	Increase, elevation
<	Less than
>	More than
≥	More than or equal to
×	Times, fold
µg	Microgramme
µmol	Micromole
bw	Body weight
CFC	Chlorofluorocarbon
CFC-11	Trichlorofluoromethane
CFC-113	Trichlorotrifluoroethane
CFC-114	1,2-Dichlorotetrafluoroethane
CFC-115	Chloropentafluoroethane
CFC-12	Dichlorodifluoromethane
d	Day
EC_{50}	Median effective concentration
F344	Fischer 344
FC	Fluorocarbon
FC-13B1	Bromotrifluoromethane, Halon 1301
FC-C318	Octafluorocyclobutane, perfluorocyclobutane
h	Hour
HCFC	Hydrochlorofluorocarbon
HCFC-123a	1,2-Dichloro-1,1,2-trifluoroethane
HCFC-141b	1,1-Dichloro-1-fluoroethane
HCFC-142b	1-Chloro-1,1-difluoroethane
hERG	Human ether-a-go-go related gene
HFC	Hydrofluorocarbon
HFC-134a	1,1,1,2-Tetrafluoroethane
HFC-227ea	1,1,1,2,3,3,3-Heptafluoropropane
HFC-245fa	1,1,1,3,3-Pentafluoropropane
kgbw	Kilogramme per body weight
K_{ir}	Inwardly rectifying potassium
LC_{50}	Median lethal concentration
LC_{L0}	Lowest lethal concentration
LD_{50}	Median lethal dose

LOAEL	Lowest observed adverse effect level
LOEL	Lowest observed effect level
MAC	Minimal alveolar anaesthetic concentration
mg	Milligramme
min	Minute
min	Minute
ml	Millilitre
mmol	Millimole
NOAEL	No observed adverse effect level
NOEL	No observed effect level
ppm	Parts per million (10^{-6})
s	Second
S9	Supernatant of centrifuged 9,000 × g liver homogenate
wk	Week
y	Year

APPENDIX A: CONVERSION FACTORS FOR VAPOUR CONCENTRATIONS IN AIR

Conversion factors for vapour concentrations in air can be calculated from the molar volume of an ideal gas at 0°C: 22.4136 litres.

$1\ mg/m^3 = 22.4136/Mw \times 1{,}013.25/P \times (273+T)/273\ ppm$ .....(Eq. B.1)
$1\ ppm = Mw/22.4136 \times P/1{,}013.25 \times 273/(273+T)\ mg/m^3$ .....(Eq. B.2)

where Mw = molecular weight, T = temperature (°C) and P = pressure (hPa).

For European standard conditions, 20°C and 1,013.25 hPa (=1 atm = 760 mm Hg), the formulae become:

$1\ mg/m^3 = 24.0556/Mw\ ppm$ .....(Eq. B.3)
$1\ ppm = Mw/24.0556\ mg/m^3$ .....(Eq. B.4)

In the USA and other countries 25°C is used, and the formulae are:

$1\ mg/m^3 = 24.4661/Mw\ ppm$ .....(Eq. B.5)
$1\ ppm = Mw/24.4661\ mg/m^3$ .....(Eq. B.6)

APPENDIX B: SOME HALOCARBONS TESTED FOR CARDIAC SENSITISATION

Halocarbon [a]	Stimulus (µg adrenaline /kgbw)	Concentration (ppm)			Reference
		NOEL	Threshold	EC_{50}	
HCFC					
HCFC-21			10,000		ECETOC, 1990
				25,000	Clark and Tinston, 1973
HCFC-31	8	50,000		100,000	Du Pont, 1973
HCFC-22	8	25,000	50,000 (2/12) [b]		Reinhardt *et al*, 1971
				140,000	Clark and Tinston, 1982
	None	600,000			Pantaleoni and Luzi, 1975a,b
	None	200,000			Belji, 1974
HCFC-123			10,000		Foll, 1976
		10,000		19,000	Trochimowicz and Mullin, 1973
HCFC-124			25,000		Mullin, 1976a
HCFC-132b		5,000	2,500		Mullin, 1976b
HCFC-141b			5,000		Mullin, 1977
		2,500	13,000		Brock *et al*, 1995
HCFC-142b	8	9,000	50,000 (5/12) [b]		Reinhardt *et al*, 1971
	Noise	25,000	800,000 (5/12) [b]		Reinhardt *et al*, 1971
	None		800,000 (1/12) [b]		Reinhardt *et al*, 1971
HCFC-225cb			19,400		Asahi Glass, 1995a
HCFC-225ca + HCFC-225cb			15,800		Asahi Glass, 1995b

APPENDIX B: SOME HALOCARBONS TESTED FOR CARDIAC SENSITISATION (cont'd)

Halocarbon [a]	Stimulus (µg adrenaline /kgbw)	Concentration (ppm)			Reference
		NOEL	Threshold	EC_{50}	
HFC					
HFC-23		800,000			Calm, 1996
HFC-32	4 - 12	350,000			Hardy and Kieran, 1992
		200,000	250,000		Calm, 1996
HFC-125	4 - 12	75,000	100,000		Kawano, 1995
HFC-134a	8	50,000	75,000		Mullin, 1979
		40,000	80,000		Hardy *et al*, 1991
HFC-143a		250,000	300,000		Calm, 1996
HFC-152a	8	50,000	150,000 (3/12) [b]		Reinhardt *et al*, 1971
HFC-218		300,000	400,000		Calm, 1996
HFC-227ea		90,000	105,000	140,000	Skaggs, 1995
HFC-236fa		100,000	150,000	200,000	Du Pont, 1994
HFC-245ea	4 - 12	10,000	20,000		Allied Signal, 1997b
HFC-245fa	4 - 12	34,000	44,000		Rusch, 1999
FC-C316	8	10,000	15,000	15,000	Du Pont, 1968

[a] See list of abbreviations. Other fluorocarbons: HCFC-21, Dichlorofluoromethane; HCFC-22, Chlorodifluoromethane; HCFC-31, Chlorofluoromethane; HFC-32, Difluoromethane; HCFC-123, Dichloro-2,2,2-trifluoroethane; HCFC-124, 1-Chloro-1,2,2,2-tetrafluoroethane; HCFC-225cb, 1,1,2,2,3-pentafluoropropane; HFC-125, Pentafluoroethane; HCFC-132b, 1,2-Dichloro-1,1-difluoroethane; HFC-143a, Trifluoroethane; HFC-152a, 1,1-Difluoroethane; HFC-218, octafluoropropane (perfluoropropane); HFC-236fa, 1,1,1,3,3,3-hexafluoropropane; HFC-245ea, 1,1,2,3,3-pentafluoropropane; HFC-245fa, 1,1,1,3,3-pentafluoropropane; FC-C316, cyclobutane, 1,2-dichloro-1,2,3,3,4,4-hexafluoro-

[b] Number of marked responses/exposed dogs

APPENDIX C: NAMING AND NUMBERING SYSTEM FOR FLUOROCARBON COMPOUNDS

The naming and numbering system currently used by industry was officially adopted as Standard 34 of the American Society of Heating, Refrigeration, and Air-conditioning Engineers (ASHRAE) on June 3, 1957 (Du Pont, 1999).

C.1 Prefixes

These prefixes are generally applicable:

FC = Fluorocarbon
CFC = Chlorofluorocarbon
HFC = Hydrofluorocarbon
PFC = Perfluorocarbon (also Perfluorocompound, Persistent Fluorinated Compound)
HFOC = Hydrofluoroether
HCFC = Hydrochlorofluorocarbon
FOC = Fluoroether.

C.2 Numbering code

The first digit from the right is the number of fluorine atoms in the molecule. The second digit from the right is one more than the number of hydrogen atoms in the molecule. The third digit from the right is one less than the number of carbon atoms in the molecule (omit if zero).

The number of chlorine atoms in the compound is calculated by subtracting the sum of fluorine and hydrogen atoms from the total atoms which can be connected to the carbon atoms. If some of the chlorine has been replaced by bromine, then the number is followed by a 'B' and the number of chlorine atoms so replaced.

The fourth digit from the right indicates the number of double bonds in the molecule, for example:

PFC-116 = 6 Fs, 0 Hs, 2 Cs and 0 Cls $\rightarrow C_2F_6$
HFC-23 = 3 Fs, 1 H, 1 C, and 0 Cls $\rightarrow CF_3H$
PFC-1216 = 6 Fs, 0 Hs, 3 Cs, 0 Cls with 1 double bond $\rightarrow C_3F_6 \rightarrow CF_2 = CF\text{-}CF_3$.

For cyclic molecules, the letter C is used before the identifying number, for example:

PFC-C318 = 8 Fs, 0 Hs, 4 Cs and 0 Cls with cyclic structure → c-C_4F_8.

For isomeric compounds, each has the same number designation, but the various isomers are indicated by a lowercase letter following the number; the letters are assigned based on the symmetry of the molecule. The most symmetrical structure has no letter, followed by the next most symmetrical isomer designated 'a', and so on. The symmetry is determined by summing the atomic weights of all atoms attached to each carbon, and comparing the two numbers. The smaller their difference, the more symmetrical the molecule. For example $C_2H_2F_4$ can have two structural isomers:

CF_2H-CF_2H, more symmetrical, HFC-134
CF_3-CFH_2, less symmetrical, HFC-134a.

C.3 Extension to 3-carbon molecules

For C3s, the isomer designation is slightly different, and uses a two-letter code. The codes below are used to determine the substituents on the central carbon, which determines the first letter of the code. The second letter in the code designates the various isomers based on symmetry, with the most symmetrical structure designated 'a', and so forth.

C.4 Letter central carbon

a = CCl_2
b = CClF
c = CF_2
d = CClH
e = CHF
f = CH_2

For example:
HFC-236fa = $C_3F_6H_2$ → Central carbon designated 'f' → CH_2 → 'a' designation → $CF_3CH_2CF_3$.

C.5 C4 and larger molecules

For 4-carbon atom and larger molecules, string together the letter designations from the above and following lists to indicate the current isomer. Always start either at the molecule's more fluorinated end or at the end needing the least number of suffix letters to assign the structure. If a digit is larger than 9, it is offset by a dash.

j = CCl_3
k = CCl_2F
l = $CClF_2$
m = CF_3
n = $CHCl_2$
o = CH_2Cl
p = CHF_2
q = CH_2F
r = CHClF
s = CH_3
t = C
x = CCl
y = CF
z = CH

Example: HFC-43-10mee = 10 Fs, 2 Hs, 5 Cs, no Cls → $C_5H_2F_{10}$

m indicates CF_3 . . . CF_3
e indicates CHF, so CF_3CHF
e indicates CHF, so $CF_3CHFCHF$
HFC-43-10mee → $CF_3CHFCHFCF_2CF_3$.

The assignment of a string of letters, to denote structural groups, is stopped when the structure is unambiguous (i.e. one does not need to call the compound HFC-43-10mee**cm**, since once one reaches ‘mee’, one knows that 5 fluorine atoms still need to be attached to the remaining two carbons, so the rest of the molecule must be $–CF_2CF_3$).

APPENDIX D: CARDIAC SENSITISATION SCORING (Chengelis, no date)

D.1: Scoring of cardiac arrhythmias for cardiac sensitisation

This table presents a scoring system for ECGs obtained while the subjects are being exposed to the test gas and then challenged with a dose of adrenaline. It is intended to be a guide tool and can not cover all possible contingencies. The final determination of a score must be made by a skilled investigator examining a subject's entire ECG record.

Score	Criteria	
0	Normal rhythm, or evidence of a typical pharmacological response to adrenaline, such as bradycardia with escape beats	
1	A single PVC (followed by a junctional beat)	Not considered evidence of cardiac sensitisation, but may occur with an adrenaline challenge without concurrent test gas exposure
2	2 to 5 PVCs in a 10 second period that are not confluent and multifocal. Not typically life threatening	Not considered evidence of sensitisation
3	6 to 10 PVCs in a 10 second period that may be multifocal but not uniformly confluent. Typically quickly resolve and not typically life threatening	Typically this score is considered equivocal, but positive, evidence of cardiac sensitisation and other factors, (e.g. the dog's base line ECG or the score obtained at a higher challenge dose of adrenaline, *etc.*) should be considered in the final interpretation
4	11 or greater PVCs that are multifocal with periods of confluency take a relatively long time to resolve and may occur in bursts between episodes of normal looking beats. Potentially life threatening	Considered unequivocal evidence of cardiac sensitisation
5	Serious, life threatening events such as ventricular tachycardia, ventricular fibrillation or flutter	Considered unequivocal evidence of cardiac sensitisation

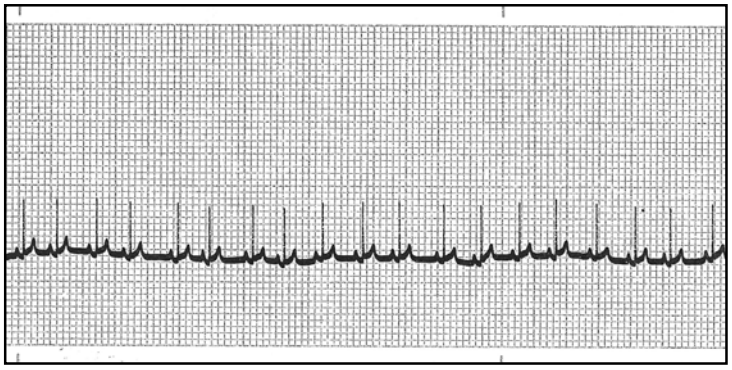
Definitions:

PVC – Premature ventricular contraction, also known ventricular extra systoles or ectopic beats

Multifocal – PVCs that originate from different parts of the ventricle and thus have a different size and shapes in the ECG

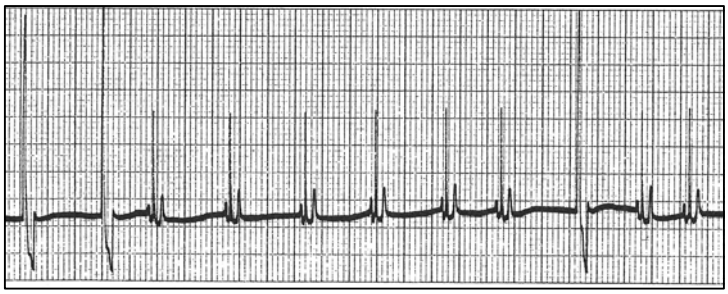
Confluency – when a PVC occurs without any apparent baseline and/or normal appearing beats between two consecutive PVCs

D.2: Samples of ECG scoring for cardiac sensitisation studies



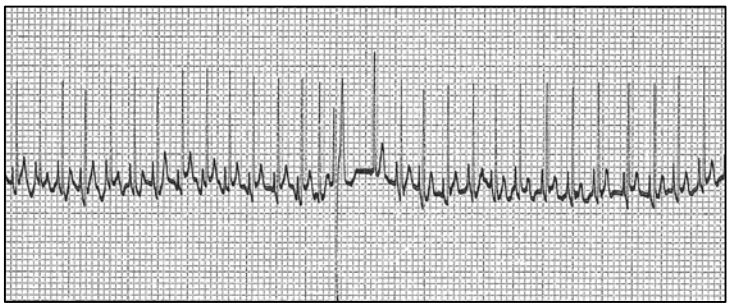
Grade 0
Normal sinus rhythm

Lead II, 10 mV/mm, 10 mm/s



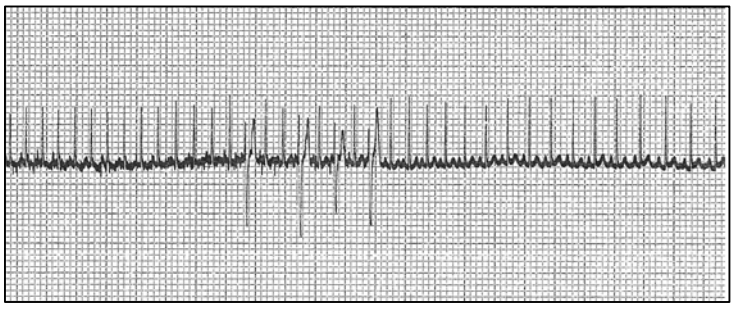
Grade 0
Junctional rhythm with escape beats

Lead II, 10 mV/mm, 10 mm/s



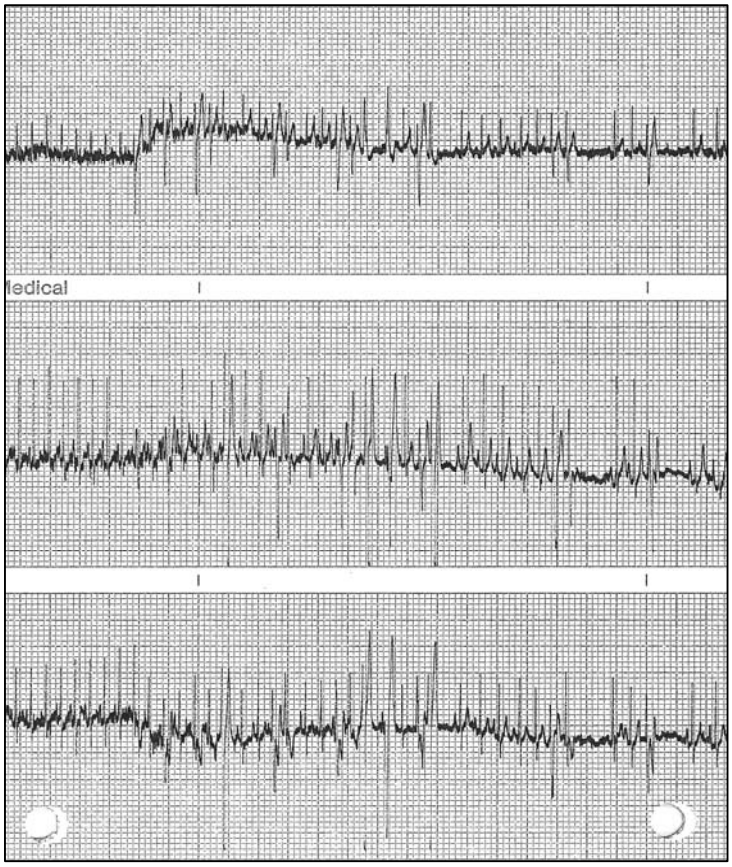
Grade 1
A single PVC followed by a junctional beat

Lead II, 10 mV/mm, 10 mm/s



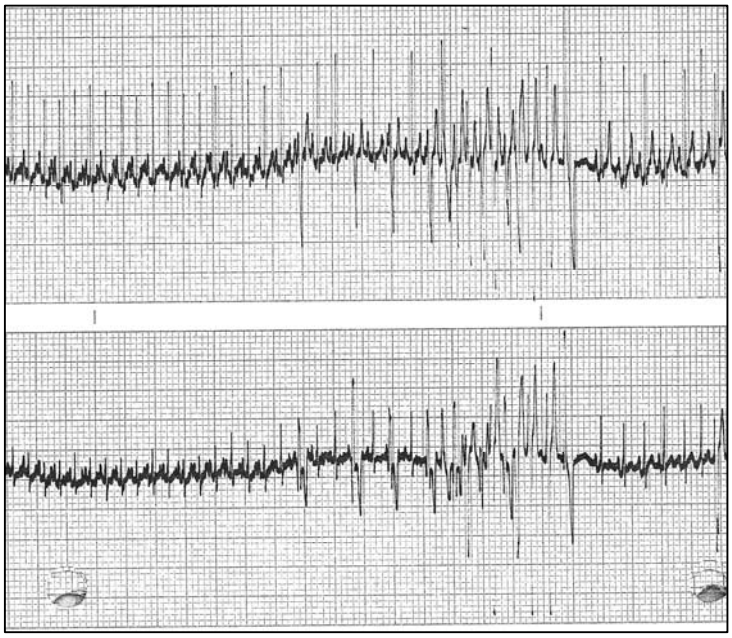
Grade 2
2 to 5 PVCs in a 10 second period with no confluence of the abnormal beats

Lead I, 10 mV/mm, 10 mm/s



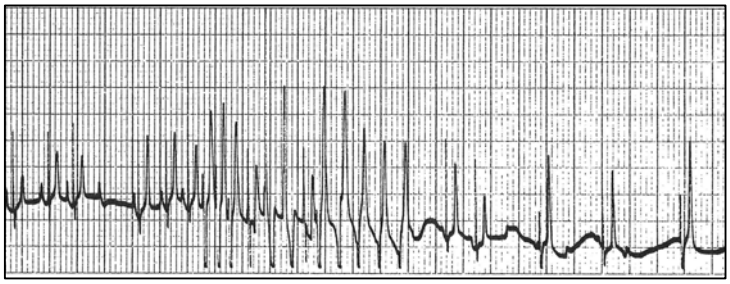
Grade 3
6 to 11 PVCs in a period of 10 seconds that maybe multifocal but with little or no confluency. This figure also demonstrates that it is often helpful to record in three leads simultaneously to obtain different views of the ECG behaviour

Lead I, II, III 10 mV/mm, 10 mm/s



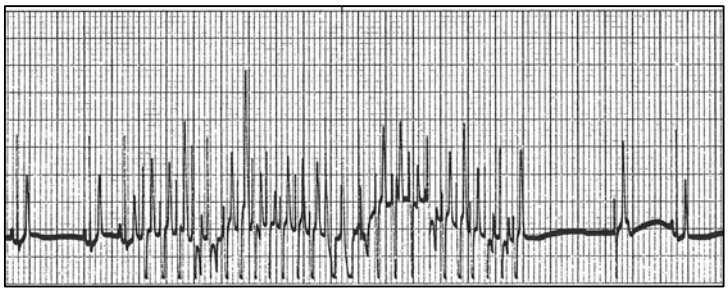
Grade 3
Another example of a grade 3 score, shown in two different leads. There are multifocal PVCs, but there are fewer than 10 abnormal beats

Lead II, III 10 mV/mm, 10 mm/s



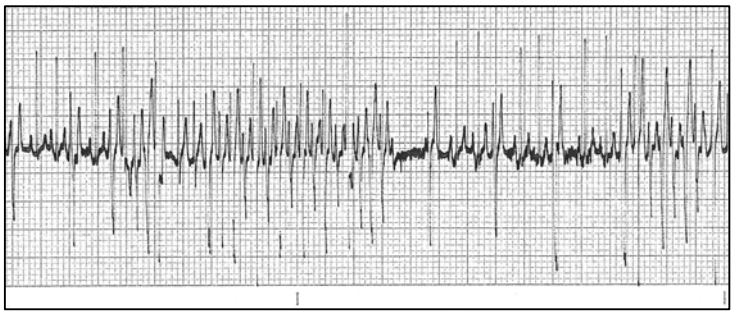
Grade 3
Another example of a grade 3 score. In comparison to grade 4 (shown below) there are fewer PVCs

Lead II, 10 mV/mm, 10 mm/s



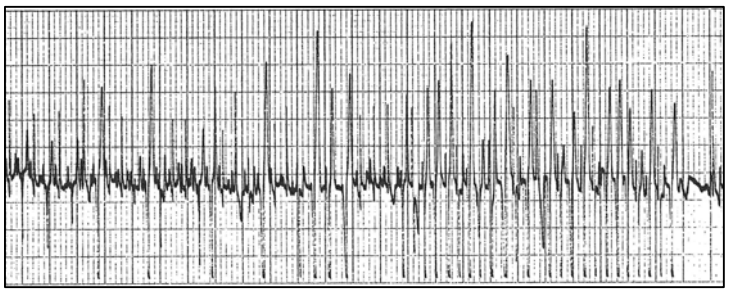
Grade 4
More than 11 PVCs in 10 seconds

Lead II, 10 mV/mm, 10 mm/s



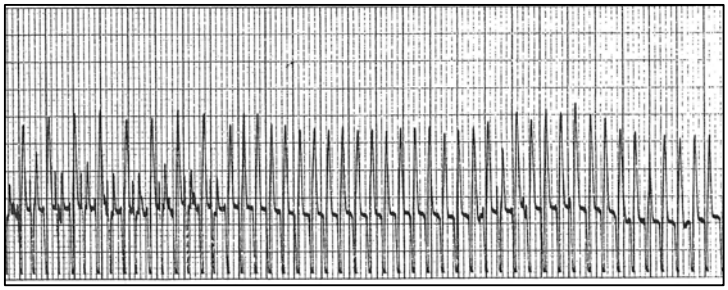
Grade 4
Bursts of tightly packed multifocal, confluent PVCs

Lead II, 10 mV/mm, 10 mm/s



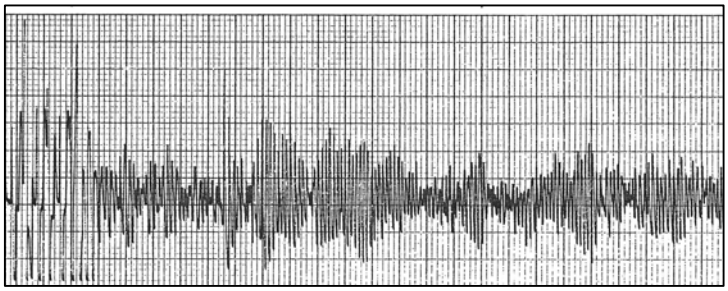
Grade 4
A run of greater than 11 multifocal, confluent PVCs in less than 10 seconds; approaching fibrillation, but returns to normal rhythm

Lead II, 10 mV/mm, 10 mm/s



Grade 5
Ventricular tachycardia

Lead II, 10 mV/mm, 10 mm/s



Grade 5
Ventricular tachycardia leading into ventricular fibrillation

Lead II, 10 mV/mm, 10 mm/s

APPENDIX E: ARRHYTHMOGENIC DOSES OF (NOR)ADRENALINE IN HUMANS AND DOGS

Species / Anaesthetic	MAC [a,b] of anaesthetic			NOEL for (nor)adrenaline (µg/kgbw)	Positive response [c]	Arrhythmogenic dose of (nor)adrenaline (µg/kgbw) [plasma concentration (µg/l)]	Administration [d]	Reference
	(Partial pressure)	(% by volume)	(mg/m^3) [e]					
Human								
Halothane	1.25				≥ 3 VES	ED_{50} ≈ 2.1	s.m.	Johnston *et al*, 1976
Isoflurane	1.25				≥ 3 VES	ED_{50} ≈ 6.7	s.m.	Johnston *et al*, 1976
	1 - 1.3			< 7.0	≥ 3 VES	ED_{50} 7.0 - 8.9	s.m.	Moore *et al*, 1993
	1 - 1.3	1.3 - 1.7	98,000 - 128,200	< 5.0	≥ 3 VES	5.0 - 9.9	s.m.	Navarro *et al*, 1994
Desflurane	1 - 1.3			< 7.0	≥ 3 VES	ED_{50} 7.0 - 8.9	s.m.	Moore *et al*, 1993
Sevoflurane	1 - 1.3	2.0 - 2.6	164,000 - 213,000	< 5.0	≥ 3 VES	5.0 - 9.9	s.m.	Navarro *et al*, 1994
Enflurane	1.25				≥ 3 VES	ED_{50} ≈ 10.9	s.m.	Johnston *et al*, 1976
Dog								
Halothane		1.0	80,700	≈ 16 [f]	VES / VT	≈ 4	i.v., slow (≈ 50 s)	Hashimoto and Hashimoto, 1972
		1.0 - 2.0	80,700 - 161,000	> 32 [f]	VF	≈ 4	i.v.	Thompson and Galysh, 1973
		1.25 - 1.5	101,000 - 121,000		≥ 20 VES	1 - 3	i.v.	Smith and Pettway, 1975

APPENDIX E: ARRHYTHMOGENIC DOSES OF (NOR)ADRENALINE IN HUMANS AND DOGS (cont'd)

Species / Anaesthetic	MAC [a,b] of anaesthetic			NOEL for (nor)adrenaline (µg/kgbw)	Positive response [c]	Arrhythmogenic dose of (nor)adrenaline (µg/kgbw) [plasma concentration (µg/l)]	Administration [d]	Reference
	(Partial pressure)	(% by volume)	(mg/m^3) [e]					
Dog (cont'd)								
	1.25	1.09	87,900		VES / VT	≈ 3.8 / ≈ 5.5	i.v., infusion (5 min)	Atlee and Malkinson, 1982
		1.1	88,700		≥ 4 VES	≈ 2.2 [≈ 39]	i.v., infusion (3 min)	Sumikawa *et al*, 1983
		0.5 - 2.0	40,300 - 161,000		≥ 4 VES	≈ 2.9 - 3.5 µg/kgbw/min	i.v., infusion (3 min)	Metz and Maze, 1985
	1.5	1.35	109,000		≥ 4 VES	≈ 6.3	i.v., infusion (3 min)	Tranquilli *et al*, 1986
		1.35	109,000		≥ 4 VES	≈ 3.4	i.v., infusion (3 min)	Venugopalan *et al*, 1989
	1.3	1.1	88,700		≥ 4 VES	≈ 2.6 µg/kgbw/min [≈ 45]	i.v., infusion (3 min)	Hayashi *et al*, 1989
		0, 0.1, 0.5, 1.0	0, 8,070, 40,300, 80,700		≥ 4 VES	≈ 10, 5.5, 3.0, 2.7 /min [≈ 200, 100, 55, 48]	i.v., infusion (3 min) [g]	Hayashi *et al*, 1991
Chloroform		0.5 - 2.0	24,400 - 97,600	> 32 [f]	VF	≈ 8	i.v.	Thompson and Galysh, 1973
		-			VF	≈ 5	i.v.	Claborn and Szabuniewicz, 1973
Enflurane		2.75	207,000		≥ 4 VES	≈ 11.4 [≈ 206]	i.v., infusion (3 min)	Sumikawa *et al*, 1983
		1.5	113,000		VES	≈ 23.7	i.v.	Aguado-Matorras, 1974

[a] Minimal alveolar anaesthetic concentration at 1 atm, where 50% of patients are insensitive to a defined noxious stimulus (equivalent to anaesthetic EC_{50} concentration)

[b] Other human MAC values (in % by volume): diethylether 1.92 , halothane 0.75, enflurane 1.68, isoflurane 1.15, desflurane 6.0, sevoflurane 2.0 (58,200, 60,500, 127,000, 86,700, 412,000 mg/m^3, respectively)

[c] VES, ventricular extrasystole; VT, (multifocal) ventricular tachycardia; VF, ventricular fibrillation

[d] By submucosal (*s.m.*) or (*i.v.*) injection or infusion as indicated

[e] Converted following Appendix A (1% = 10,000 ppm)

[f] Without anaesthetic

[g] Basal adrenaline ≈ 0.4 µg/l plasma

APPENDIX F: POTENCY OF VOLATILE ANAESTHETICS AT CARDIAC ION CHANNELS / TARGETS

Anaesthetic (MAC[a], µmol/l)										**Remark**	**Reference**
Halothane (≈ 210)		**Enflurane** (≈ 520)		**Isoflurane** (≈ 310)		**Sevoflurane** (≈ 350)		**Chloroform, other** (≈ 860)			
(µmol/l)	**Current[b]** (Vh, mV)	**(µmol/l)**	**Current[b]**	**(µmol/l)**	**Current[b]** (Vh, mV)	**(µmol/l)**	**Current[b]** (Vh, mV)	**(µmol/l)**	**Current[b]**		
Na^+											
630	↓ 60% (–80)									Steady-state inactivation –8 mV, delayed recovery	Ikemoto *et al*, 1986
1,200	↓ 24/42% (–110/–80)			1,000	↓ 11% (–110)	1,200	↓ 11% (–110)			Left shift of steady-state inactivation, delayed recovery	Weigt *et al*, 1997a, 1997
470	↓ 21/67% (–120/–90)			540	↓ 15/56% (–120/–90)					Delayed recovery	Stadnicka *et al*, 1999
450	↓ 25% (–100)			560	↓ 36% (–100)					Persistent I_{Na} !!	Eskinder *et al*, 1993
						1,000	↓ 20% at 35°C (–70)				Kang *et al*, 2006

APPENDIX F: POTENCY OF VOLATILE ANAESTHETICS AT CARDIAC ION CHANNELS / TARGETS (cont'd)

Anaesthetic (MAC[a], µmol/l)										**Remark**	**Reference**
Halothane (≈ 210)		**Enflurane** (≈ 520)		**Isoflurane** (≈ 310)		**Sevoflurane** (≈ 350)		**Chloroform, other** (≈ 860)			
(µmol/l)	**Current[b]** (Vh, mV)	**(µmol/l)**	**Current[b]**	**(µmol/l)**	**Current[b]** (Vh, mV)	**(µmol/l)**	**Current[b]** (Vh, mV)	**(µmol/l)**	**Current[b]**		
Ca^{2+}, L-type											
500	↓ 30%	1,050	↓ 30%	750	↓ 30%					^{3}H-nitrendipine binding	Nakao *et al*, 1989
400	↓ 31%	800	↓ 32%	610	↓ 38%						Bosnjak *et al*, 1991
450	↓ 34%	650	↓ 32%	540	↓ 33%						Eskinder *et al*, 1991
		1,460	↓ 31%								Puttick and Terrar, 1992
900	↓ 52%			800	↓ 38%					Slowly inactivating I_{Ca} (cardiac action potential wave)	Pancrazio, 1996
						1,000	↓ 30%				Kang *et al*, 2006
600	↓ 43% at 30°C			600	↓ 24% at 30°C	600	↓ 12% at 30°C			Subepicardial rat myocytes	Rithalia *et al*, 2004

APPENDIX F: POTENCY OF VOLATILE ANAESTHETICS AT CARDIAC ION CHANNELS / TARGETS (cont'd)

Anaesthetic (MAC [a], µmol/l)										**Remark**	**Reference**
Halothane (≈ 210)		**Enflurane** (≈ 520)		**Isoflurane** (≈ 310)		**Sevoflurane** (≈ 350)		**Chloroform, other** (≈ 860)			
(µmol/l)	**Current [b]** (Vh, mV)	**(µmol/l)**	**Current [b]**	**(µmol/l)**	**Current [b]** (Vh, mV)	**(µmol/l)**	**Current [b]** (Vh, mV)	**(µmol/l)**	**Current [b]**		
Ca^{2+}, T-type											
450	↓ 33%	650	↓ 30%	540	↓ 37%						Eskinder *et al*, 1991
K, hERG [d] / I_{Kr}											
				300	↓ 21%						Suzuki *et al*, 2003
						650	↓ 27%			hERG in *Xenopus* oocytes	Yamada *et al*, 2006
								5	↓ 50%		Scholz *et al*, 2006
K^+, I_{Ks}											
				510	↓ 33%	540	↓ 27%			KvLQT1/minK in *Xenopus* oocytes	Chen *et al*, 2002
				300	↓ 50%						Suzuki *et al*, 2003
1,070	↓ 50%					380	↓ 50%				Shibata *et al*, 2004
						1,300	↓ 50%				Kang *et al*, 2006

APPENDIX F: POTENCY OF VOLATILE ANAESTHETICS AT CARDIAC ION CHANNELS / TARGETS (cont'd)

Anaesthetic (MAC [a], µmol/l)										**Remark**	**Reference**
Halothane (≈ 210)		**Enflurane** (≈ 520)		**Isoflurane** (≈ 310)		**Sevoflurane** (≈ 350)		**Chloroform, other** (≈ 860)			
(µmol/l)	**Current [b]** (Vh, mV)	**(µmol/l)**	**Current [b]**	**(µmol/l)**	**Current [b]** (Vh, mV)	**(µmol/l)**	**Current [b]** (Vh, mV)	**(µmol/l)**	**Current [b]**		
K^+, I_{to}											
						1,000	↓ 40%			Inactivation time constant	Kang *et al*, 2006
600	↓ 8% at 30°C			600	↓ 7% at 30°C	600	↓ 2% at 30°C			Subepicardial rat myocytes	Rithalia *et al*, 2004
1,100	↓ 50% at 30°C									Accelerated inactivation, steady-state inactivation – 16 mV	Davies *et al*, 2000
K+, inward rectifier											
300	↑ 38% basal, ↓ stimulated									GIRK1/GIRK4 (= $K_{ir}3.x$) in *Xenopus* oocytes	Weigl and Schreib-Mayer, 2001
1,870	↓ 22%			1,810	↓ 24%					$K_{ir}2.x$, slope conductance negative to E_K	Stadnicka *et al*, 2000

APPENDIX F: POTENCY OF VOLATILE ANAESTHETICS AT CARDIAC ION CHANNELS / TARGETS (cont'd)

Anaesthetic (MAC [a], µmol/l)										**Remark**	**Reference**
Halothane (≈ 210)		**Enflurane** (≈ 520)		**Isoflurane** (≈ 310)		**Sevoflurane** (≈ 350)		**Chloroform, other** (≈ 860)			
(µmol/l)	**Current [b]** (Vh, mV)	**(µmol/l)**	**Current [b]**	**(µmol/l)**	**Current [b]** (Vh, mV)	**(µmol/l)**	**Current [b]** (Vh, mV)	**(µmol/l)**	**Current [b]**		
APD_{90} [c]											
1,060	↓ 40%									Triangulation !! (guinea-pig ventricular myocytes)	Hirota *et al*, 1989
1,200	↓ 30%									No triangulation (guinea-pig ventricular myocytes)	Terrenoire *et al*, 2000
650	↓ 15% in dog Purkinje fibre									Triangulation !! (proximal), little distal effect	Turner *et al*, 1990
1,150	↓ 21% in dog Purkinje fibre									↓ effective refractory period, triangulation !, little effects in	Freeman *et al*, 1991, 1991b
Conduction											
710	↓ 13% in dog Purkinje fibre									↓ conduction at Purkinje fibre-papillary muscle junction	Freeman *et al*, 1991
400	No effect in Purkinje fibre									Conduction ↓ at Purkinje fibre-papillary muscle	Vodanovic *et al*, 1997
400	↓ 3% in Purkinje fibre									Potentiated by adrenaline, lidocaine, octanol, *etc*	Kulier *et al*, 2000

APPENDIX F: POTENCY OF VOLATILE ANAESTHETICS AT CARDIAC ION CHANNELS / TARGETS (cont'd)

Anaesthetic (MAC [a], µmol/l)										**Remark**	**Reference**
Halothane (≈ 210)		**Enflurane** (≈ 520)		**Isoflurane** (≈ 310)		**Sevoflurane** (≈ 350)		**Chloroform, other** (≈ 860)			
(µmol/l)	**Current [b]** (Vh, mV)	**(µmol/l)**	**Current [b]**	**(µmol/l)**	**Current [b]** (Vh, mV)	**(µmol/l)**	**Current [b]** (Vh, mV)	**(µmol/l)**	**Current [b]**		
Intercellular coupling											
≥ 750	Inhibition			1,200	Inhibition						Terrar and Victory, 1988
3,000	Total inhibition							500 octanol 1,000 heptanol	Block		Niggli *et al*, 1989

[a] Minimal alveolar anaesthetic concentration at 1 atm, where 50% of patients are insensitive to a defined noxious stimulus (equivalent to anaesthetic EC_{50} concentration). MAC values listed are mean values of multiple MAC determinations in mammals and humans (1 atm, 37°C) from various references (Franks and Lieb, 1993, 1994; Krasowski and Harrison, 1999). Anaesthetic concentrations listed in the remainder of the table are concentrations in water at 35 to 37°C

[b] De-/increase at room temperature (20 - 22°C) unless indicated otherwise; Vh, holding potential

[c] Action potential duration at 90% repolarisation

Note: Anaesthetic concentrations at room temperature can be estimated for physiological temperatures where necessary according to the algorithms provided by Franks and Lieb (1993). As a rule of thumb, aqueous EC_{50} concentrations at room temperature are about 20 to 25% lower than those at 37°C

MEMBERS OF THE TASK FORCE

D. Farrar (Chairman)	Ineos Chlor UK - Runcorn
M. Binaglia	Solvay B - Brussels
C. Chengelis	WIL Research Laboratory USA - Ohio
C. Hardy	Huntingdon Life Sciences UK - Cambridgeshire
H. Himmel	Bayer HealthCare D - Wuppertal
G. Jepson	Du Pont De Nemours USA - Newark
C. Reinhardt	Consultant USA - Pennsylvania
G. Rusch	Honeywell USA - Morristown
B. Schmit	Solvay B - Brussels
H. Vrijhof (Secretary)	ECETOC B - Brussels

MEMBERS OF THE SCIENTIFIC COMMITTEE

J. Doe (Chairman) [a] Head of Health Assessment	Syngenta UK - Bracknell
R. Bars Team Leader, Toxicology Research	Bayer CropScience F - Sophia Antipolis
P. Calow Professor, Department of Environmental, Social and Spatial Change	University of Roskilde DK - Roskilde
W. de Wolf Director of Health and Environment Sciences	Du Pont B - Brussels
D. Farrar Occupational Health Business Manager	Ineos Chlor UK - Runcorn
A. Flückiger [a] Head of Corporate Health Protection	F. Hoffmann-La Roche CH - Basel
H. Greim Director, Institute of Toxicology and Environmental Hygiene	Technical University Munich D - Munich
F. Lewis Head of Environmental Safety - EAME	Syngenta UK - Jealott's Hill, Bracknell
G. Malinverno European Public Affairs Manager	Solvay B - Brussels
S. Marshall Environmental Science Leader	Unilever- SEAC UK - Bedford
C. Money Industrial Hygiene Adviser - Europe	ExxonMobil B - Brussels
D. Owen Scientific and Regulatory Manager	Shell Chemicals UK - London

[a] Steward responsible for primary peer review

MEMBERS OF THE SCIENTIFIC COMMITTEE (cont'd)

M. Pemberton Global Product Integrity Manager	Lucite International UK - Wilmslow
C. Rodriguez Principal Toxicologist, Corporate Central Product Safety	Procter & Gamble B - Strombeek-Bever
D. Salvito Environmental Scientist	RIFM USA - Woodcliff Lake
G. Swaen Senior Epidemiologist	Dow Europe NL - Terneuzen
J. Tolls Director Environmental Safety Assessment	Henkel KgaA D - Düsseldorf
S. van der Vies Professor of Biochemistry	Vrije Universiteit Amsterdam NL - Amsterdam
B. van Ravenzwaay Director, Experimental Toxicology and Ecology	BASF D - Ludwigshafen
E. von Keutz Head of Toxicology	Bayer Healthcare D - Wuppertal
H.-J. Wiegand Head, Product Safety Department	Degussa D - Essen