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Dear Dr. Masten:

On behalf of the U. S. Food and Drug Administration, I nominate acrylamide, and its principal metabolite glycidamide, to the National Toxicology Program as FDA's priority chemical nomination for Fiscal Year 2003. Under the NTP Scientific Issues Initiative, I also nominate drugs positive for QT Interval Prolongation/Induction of *Torsade* Proarrhythmia, under conditions of therapeutic use.

Acrylamide and Glycidamide

The presence of acrylamide in processed starchy foods first came to the attention of the FDA on April 24, 2002, when researchers at the Swedish National Food Administration and Stockholm University reported finding acrylamide in a variety of fried and oven-baked foods. The presence of acrylamide in such foods was associated with standard high-temperature cooking processes used in their preparation. Subsequent to the Swedish report, the FAO/WHO scheduled a Consultation on the Health Implications of Acrylamide in Food on June 25-27, 2002 in Geneva, Switzerland. The FAO/WHO conclusions recognized the potential carcinogenic risk to the general public from consumption of starchy fried and baked foods containing acrylamide, and listed several research areas that should be investigated (Attachment 1). Since the Swedish report, similar findings have been reported by Norway, the United Kingdom, Switzerland, Japan, and by the FDA.

It is known that following oral administration to rodents, absorption and elimination of acrylamide is rapid and distribution to tissues is extensive. Acrylamide is converted to a reactive epoxide metabolite, glycidamide, primarily through the action of the CYP 2E1 isozyme. The elimination half-life for glycidamide is slightly shorter than that for acrylamide. Both acrylamide and glycidamide react with nucleophilic amino acids in hemoglobin, notably the N-terminal valine and cysteine residues, reactions that have been used for assessing internal exposures in rodents and humans. In addition, both form glutathione conjugates that are excreted in the urine as mercapturic acid derivatives in rodents and humans. Glycidamide and acrylamide form hemoglobin adducts in rodents at ratios (acrylamide to glycidamide) similar to those observed in humans, which suggests that rodents are appropriate models for assessing exposures to glycidamide derived from acrylamide metabolism. Moreover, both acrylamide and glycidamide react with DNA (glycidamide much more rapidly) to form DNA adducts. While acrylamide is listed as 'reasonably anticipated to be a human carcinogen' in the NTP's Report on Carcinogens, the FDA requires a properly designed (dose response considerations and accounting for the food matrix through which humans are exposed), well-conducted, GLP-compliant bioassay. Results from such studies will provide the agency with sound scientific data by which more accurate risk assessments can be conducted (Attachment 2). Additional information on acrylamide may be found at the FAO/WHO Acrylamide in Foods Network, <http://www.acrylamide-food.org>.

The FDA held a federal interagency acrylamide meeting at FDA's Center for Food Safety and Applied Nutrition in College Park, Maryland on September 24, 2002 to review ongoing research initiatives, to provide suggestions regarding bio-monitoring, and to identify research gaps. Results from those discussions supported the FDA plan to nominate both acrylamide and glycidamide to the NTP for extensive toxicological testing (Attachments 3 and 4). The agency also held a public meeting on September 30, 2002 to solicit public comments and to present the FDA action plan for acrylamide. Public comments also supported the FDA Acrylamide Action Plan. Transcripts and slide presentations from that meeting can be viewed at <http://www.cfsan.fda.gov/~dms/acryagen.html> [Center for Food Safety and Applied Nutrition contact person: Dr. Richard A. Canady].

Drugs Positive for QT Interval Prolongation / Induction of *Torsade Proarrhythmia*

QT interval prolongation and an associated severe life-threatening ventricular arrhythmia, *torsade de pointes*, is a high priority cause for concern in drug development and regulatory safety evaluation. Of the drugs recently removed from the U.S. market, one of the most common causes has been QT interval-related cardiac toxicity. For example, the non-sedating antihistamines, Terfenadine and Astemizole, and the pro-kinetic agent, cisapride, were removed from the U.S. market because of this cardiac toxicity. Additionally, the non-sedating antihistamine, Ebastine, and the antipsychotic agent, Sertindole, were denied access to the U.S. market for this reason. Finally, the antipsychotic agent, Ziprasidone, and the fluoroquinolone antibiotic, Moxifloxacin, two new drugs that prolonged QT interval in clinical trials, are labeled with severe warnings for this toxicity. Clearly, this cardiac toxicity cuts across therapeutic indications, and is therefore a general problem in drug development. Additionally, in the majority of the above cases, this cardiac toxicity was discovered either after approval during clinical use or in late stage clinical trials rather than in early drug development, with significant resultant difficulties.

Given the medical and economic consequences of this issue, the International Conference on Harmonization established an Expert Working Group to draft guidance recommending the incorporation into drug development of preclinical models predictive of QT interval prolongation and proarrhythmia. This draft guidance, ICH S7B, was signed as a Step 2 draft document in February 2002, and published for comment in the Federal Register in June, 2002 (Attachment 5). ICH S7B recommends a testing strategy comprised of both *in vitro* and *in vivo* assays considered likely to be predictive for drug-induced QT interval prolongation and proarrhythmia. Among these assays is an ionic current assay previously shown to be sensitive to known QT prolonging and proarrhythmic drugs, a ventricular repolarization assay that integrates on a cellular level test agents' effects on several ionic currents, and a conscious canine model that integrates test agents' effects on a whole animal level. These models, while likely to be predictive for QT interval prolongation and proarrhythmia, have not been rigorously evaluated for their predictability. The general consensus is that the dog is likely the best *in vivo* model but we do not have the data to sufficiently anchor its performance against drugs that we have had clinical experience with.

To address this deficiency, ILSI/HESI, with support from PhRMA and FDA, is evaluating the sensitivity and specificity of the ICH S7B recommended *in vitro* ionic current and ventricular repolarization assays, and an *in vivo* conscious canine telemetry model embraced by the pharmaceutical industry. Test agents to be evaluated in this exercise include non-antiarrhythmic drugs that are clearly positive for QT interval prolongation and proarrhythmia in humans, as well as those considered to be clearly negative for these liabilities under conditions of clinical use. However, ILSI/HESI is evaluating only a limited subset of non-antiarrhythmic drugs (those that are off patent and/or not presently marketed) recommended to be tested by FDA. The FDA can not require that sponsors evaluate the performance of their products in these model systems using problematic drugs that are presently marketed, since the risk is already known from clinical data and FDA does not have the regulatory authority to require preclinical testing under such conditions. The FDA also does not have the regulatory authority to require preclinical testing of non-problematic, negative control drugs. The level of clinical risk of QT prolongation and arrhythmia is fairly well accepted for the list of drugs named below. For those agents that are still marketed the regulatory judgement is that benefit outweighs risk when appropriate product labeling is provided for prescribing physicians to make decisions on a patient-by-patient basis. How these agents will compare to one another in a single set of well-designed test models using a shared protocol has not been investigated. A critical data gap exists in our knowledge of how well the *in vivo* dog model will perform in its ability to discriminate problematic from non-problematic agents and the transitional gray area between.

To fill these data gaps, the FDA recently collaborated with Georgetown University to evaluate several remaining torsadogenic drugs on the FDA lists. Due to priorities and economics, FDA/Georgetown chose to evaluate these drugs in the ionic current assay considered likely to be predictive for QT interval prolongation and proarrhythmia rather than in the more costly *in vivo* model. These data are available and are a useful complement to the ILSI/HESI data initiative, but have not yet been published. While the *in vitro* tests are clearly capable of identifying hazard potential, they are less capable of evaluating risk. For example, it is difficult to correlate *in vitro* and *in vivo* concentrations, only the parent compound is evaluated *in vitro*, and *in vitro* exposure times are limited compared to *in vivo* exposures. In contrast, *in vivo* assays, such as the conscious canine telemetry model, can be used to evaluate risk by enabling assessments of safety margins based on relative drug and metabolite exposures. *In vivo* assays can also consider risk in the perspective of additional concurrent toxicities and pharmacological properties of a novel test agent.

In order to address both sensitivity and specificity of an experimental model it is important to evaluate both positive and negative controls. Additionally, the broadest range and most complete set of test agents should be evaluated to minimize error and bias. Finally, since results of the preclinical assays will influence the extent and design of clinical evaluation of drugs for QT interval prolongation, FDA believes it important to know whether the suggested nonclinical assays would detect drugs that have been shown to prolong QT interval in clinical trials, but for which clear evidence of proarrhythmia is lacking.

Consequently the FDA requests that the NTP evaluate both problematic and non-problematic drugs in the conscious, canine telemetry model, in order to better establish the sensitivity and specificity of this *in vivo* model system for evaluating the property of a test agent to prolong QT interval at relevant exposures in humans. Unlike other proposals previously nominated by FDA to NTP, in this proposal the FDA requests that a model system, namely the conscious canine telemetry model, be more rigorously evaluated using various drugs named below. The FDA is in significant need of a clearer definition of the strength and limitations of this model and future performance characteristics with unknown candidates, rather than more insight into the safety concerns *per se* of the listed compounds [Center for Drug Evaluation and Research contact person: Dr. Frank D. Sistare].



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Attachments

1. Summary Report, FAO/WHO Consultation on the Health Implications of Acrylamide in Food, June 2002
2. Introduction to the Environmental Toxicology of Acrylamide
3. Acrylamide Nomination Dossier, Oct 30, 2002
4. Summary of Discussions from Breakout Groups
5. Federal Interagency Acrylamide Research Meeting, Summary and Research Needs, September 2002
6. Draft ICH Consensus Guideline, Safety Pharmacology Studies for Assessing the Potential for Delayed Ventricular Repolarization (Qt Interval Prolongation) by Human Pharmaceuticals



Table 1: The FDA generated lists of torsadogenic and non-torsadogenic drugs. The named drugs were selected based on FDA clinical information and represent those that were tested in the *in vitro* ionic current assay. Of this broad list of compounds several were selected because they were chosen by ILSI/HESI (for overlap purposes), and others that are not being evaluated by ILSI/HESI. Ideally all agents should be tested but if funding limitations do not allow testing of the complete list, the agents could be prioritized and further limited.

Drugs Believed to be Pro-arrhythmic in Humans Under Conditions of Use*	ILSI/HESI Ionic Current Assay/ Conscious Canine Telemetry Model	FDA /Georgetown Ionic Current Assay	NTP Conscious Canine Telemetry Model
Amiodarone^			
Arsenic trioxide		+	+
Bepriidil	+		
Droperidol, iv			+
Disopyramide^			
Dofetilide			+
Domperidone, iv			+
Erythromycin, iv			
Clarithromycin, iv			
Halofantrine, iv			+
Haloperidol, iv	+	+	+
Ibutilide^			
Levomethadyl (Orlaam)		+	+
Pentamidine, iv		+	+
Pimozide	+		+
Procainamide^			
NAPA^			
Propafenone		+	+
Quinidine^			
Sotalol			+
Sparfloxacin			+
Thioridazine	+	+	+
Aprindine^			
Astemizole			+
Cisapride	+		+
Grepafloxacin			+
Lidoflazine		+	+
Prenylamine		+	+
Terfenadine	+	+	+
Terodiline	+		+
Sertindole		+	+
Probuocol		+ not evaluable <i>in vitro</i>	+
Ziprasidone#			+
Moxifloxacin#			+

*The above lists are not comprehensive.

^ Antiarrhythmic drugs not evaluated

Drugs that prolong QT, but were not demonstrated to induce proarrhythmia in clinical trials. These drugs were not included in the FDA list of torsadogenic drugs.

Some Drugs Not Believed to be Pro-arrhythmic in Humans Under Conditions of Use*	ILSI/HESI Ionic Current Assay/ Conscious Canine Telemetry Model	FDA /Georgetown Ionic Current Assay	NTP Conscious Canine Telemetry Model
Olanzapine			+
Quetiapine			+
Risperidone			+
Cetirizine			+
Chlorpheniramine		+	+
Fexofenadine			+
Loratadine		+	+
Pyrilamine		+	+
Amoxicillin	+		
Ciprofloxacin			
Verapamil	+		
Diltiazem			+
Nifedipine			
Aspirin	+		
Captopril	+		
Angiotensin II blocker			
Propranolol	+		
Benzodiazepine			
Cimetidine		+	+
Lovastatin		+	+

*The above lists are not comprehensive.

INTERNATIONAL CONFERENCE ON HARMONISATION OF TECHNICAL
REQUIREMENTS FOR REGISTRATION OF PHARMACEUTICALS FOR HUMAN
USE

DRAFT CONSENSUS GUIDELINE

**SAFETY PHARMACOLOGY STUDIES FOR ASSESSING THE
POTENTIAL FOR DELAYED VENTRICULAR
REPOLARIZATION
(QT INTERVAL PROLONGATION)
BY HUMAN PHARMACEUTICALS**

Released for Consultation
at *Step 2* of the ICH Process
on 7 February 2002
by the ICH Steering Committee

At Step 2 of the ICH Process, a consensus draft text or guideline, agreed by the appropriate ICH Expert Working Group, is transmitted by the ICH Steering Committee to the regulatory authorities of the three ICH regions (the European Union, Japan and the USA) for internal and external consultation, according to national or regional procedures.

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Draft ICH Consensus Guideline**

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SAFETY PHARMACOLOGY STUDIES FOR ASSESSING THE POTENTIAL FOR DELAYED VENTRICULAR REPOLARIZATION (QT INTERVAL PROLONGATION) BY HUMAN PHARMACEUTICALS

1. INTRODUCTION

This draft guideline is based upon the best currently available science. However, it addresses a field of research that is in a state of rapid evolution, and interested parties are encouraged to submit data with protocols and other information for use in developing the final guideline.

1.1 Objectives of the Guideline

This guideline was developed to: 1) protect clinical trial participants and patients receiving marketed products from delayed repolarization-associated ventricular tachycardia, torsade de pointes, and lethal arrhythmia resulting from administration of pharmaceuticals, 2) recommend study types and timing of studies in relation to clinical development, and 3) provide general principles and information on currently available nonclinical methodologies to identify the potential hazard and assess the risk of QT interval prolongation by a pharmaceutical.

1.2 Background

The QT interval (time from the beginning of the QRS complex to the end of the T wave) of the electrocardiogram (ECG) is a measure of the duration of ventricular depolarization and repolarization. QT interval prolongation can be congenital or acquired (e.g., pharmaceutical-induced). When the QT interval is prolonged, there is an increased risk of ventricular tachyarrhythmia, including torsade de pointes, particularly when combined with other risk factors (e.g., hypokalemia, structural heart disease, bradycardia). Thus, much emphasis has been placed on the potential proarrhythmic effects of pharmaceuticals that are associated with QT interval prolongation.

Results from nonclinical studies to address a potential for QT interval prolongation contribute to the design and interpretation of clinical investigations. Therefore, careful nonclinical investigation of the ability of pharmaceuticals to prolong the QT interval should be carried out to assess potential risk for humans.

Ventricular repolarization, determined by the duration of the cardiac action potential, is a complex physiological process. It is the net result of the activities of many membrane ion channels and transporters. Under physiological conditions, the functions of these ion channels and transporters are highly interdependent. The activity of each ion channel or transporter is affected by multiple factors that are controlled by the other ion transporters including, but not limited to, intracellular and extracellular ion concentrations, membrane potential, cell-to-cell electrical coupling, heart rate, and autonomic nervous system activity. The metabolic state (e.g., acid-base balance) and location and type of cardiac cell are also important. The human ventricular action potential consists of five sequential phases:

- phase 0: The upstroke of the action potential is primarily a consequence of a rapid, transient influx of Na^+ (I_{Na}) through Na^+ channels.
- phase 1: The termination of the upstroke of the action potential and early repolarization phase result from the inactivation of Na^+ channels and the transient efflux of K^+ (I_{to}) through K^+ channels.

- phase 2: The plateau of the action potential is a reflection of a balance between the influx of Ca^{2+} (I_{Ca}) through L-type Ca^{2+} channels and outward repolarizing K^+ currents.
- phase 3: The sustained downward stroke of the action potential and the late repolarization phase result from the efflux of K^+ (I_{Kr} and I_{Ks}) through delayed rectifier K^+ channels.
- phase 4: The resting potential is maintained by the inward rectifier K^+ current (I_{K1}).

In theory, prolongation of the action potential can result from decreased inactivation of the inward Na^+ or Ca^{2+} currents, increased activation of the Ca^{2+} current, or inhibition of one or more of the outward K^+ currents. The rapidly and slowly activating components of the delayed rectifier potassium current, I_{Kr} and I_{Ks} , seem to have the most influential role in determining the duration of the action potential and thus the QT interval. The human ether-à-go-go-related gene (hERG) and KvLQT1 gene encode pore-forming proteins that are thought to represent the α -subunits of the human potassium channels responsible for I_{Kr} and I_{Ks} , respectively. These α -subunit proteins can form hetero-oligomeric complexes with auxiliary β -subunits (i.e. MiRP and MinK gene products), which have been speculated to modulate the gating properties of the channel proteins. The most common mechanism of QT interval prolongation by pharmaceuticals appears to be inhibition of the delayed rectifier potassium channel that is responsible for I_{Kr} .

1.3 Scope of the Guideline

This guideline extends and complements the “ICH Guideline on Safety Pharmacology Studies for Human Pharmaceuticals” (ICH S7A). The pharmaceuticals to be tested are described in ICH S7A. Principles and recommendations concerning study design, including concentration and dose ranges, described in ICH S7A also apply to the studies conducted in accordance with the present guideline.

1.4 General Principles

The choice of assays and data used to identify the hazard of QT interval prolongation for a test substance should be scientifically based and flexible. *In vitro* and *in vivo* assays are complementary approaches; therefore, according to current understanding, more than one type of assay should be conducted.

The investigational approach and conclusions concerning acceptable risk can be individualized for the test substance, depending on its pharmacodynamic, pharmacokinetic, and safety profiles and its clinical indication.

The development of new technologies and methodologies is encouraged, with the goal of identifying methods that are scientifically valid, practical, robust, and predictive of human risk.

Carefully designed clinical studies can assess whether the potential risk for delayed ventricular repolarization identified in nonclinical studies is relevant to humans.

2. GUIDELINE

2.1 Objectives of Studies

The objectives of these studies are to: 1) identify potential of a test substance and its metabolites to delay ventricular repolarization, and 2) relate the extent of delayed ventricular repolarization to the concentrations of the parent substance and its metabolites. This information, along with information from pharmacodynamic and toxicology studies, can be used to estimate human risk.

2.2 General Considerations for Selection and Design of Studies

Nonclinical methodologies can address four electrophysiological levels of integration that can manifest as delayed ventricular repolarization and potential sequelae. These levels include:

- Ionic currents measured in isolated animal or human cardiac myocytes, cultured cardiac cell lines, or heterologous expression systems for cloned human ion channels.
- Action potential parameters in isolated cardiac preparations or specific electrophysiology parameters indicative of action potential duration in anesthetized animals
- ECG parameters measured in conscious or anesthetized animals.
- Proarrhythmic effects measured in isolated cardiac preparations or animals.

As indicated above, these four functional levels can be investigated by *in vitro* and/or *in vivo* methods. Although proarrhythmia models are listed, there is insufficient information at this time to recommend these studies for risk assessment (see section 3.4.4). Findings from the first three functional levels discussed above are considered useful and complementary.

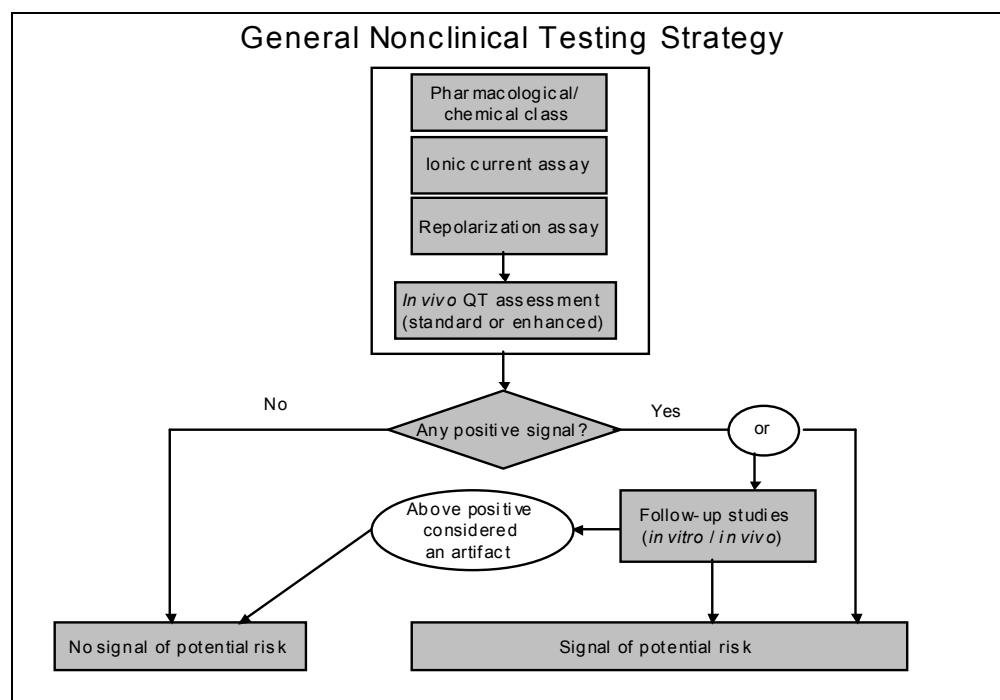
In vitro electrophysiology studies can explore potential cellular mechanisms that may not be evident from *in vivo* data. Changes in other cardiovascular parameters or effects on multiple ion channels can complicate interpretation of data. Complementary assessments in other systems can address this issue. While inhibition of I_{Kr} is thought to be the most common cellular mechanism responsible for pharmaceutical-induced prolongation of QT interval in humans, delay of repolarization through actions on other ion channels is possible.

Studies of action potential parameters provide information on the integrated activity of multiple ion channels in the heart, while *in vivo* ECG evaluations provide information on conduction properties and non-cardiac influences (e.g., autonomic nervous system tone). Thus, experimental models that possess the full complement of mechanisms can be more informative with regard to the clinical situation. Carefully designed and conducted *in vivo* studies also allow evaluation of metabolites and can enable estimation of safety margins. When signals of potential risk are generated in these integrated models, results should be correlated with results from *in vitro* studies. It should be noted that the choice of species and experimental conditions can affect the ability to extrapolate nonclinical results to the clinical setting. Despite this caveat, data from *in vivo* models are an important component in the assessment of the potential for QT interval prolongation in humans.

2.3 General Recommendations for Testing Strategies in Nonclinical Studies and Implications of the Study Results

2.3.1 Recommended Nonclinical Testing Strategy

The following section describes a general nonclinical testing strategy and an evaluation approach for assessing signals of potential risk for QT interval prolongation that are pragmatic and based on currently available information. The figure illustrates the component elements of the testing strategy, but not specific test systems or their designs. Specific test systems are described in section 3.



As illustrated, the following information should generally be provided:

1. Evaluation of whether the test substance belongs to a pharmacological/chemical class known to prolong QT interval in humans (see below)
2. Results from an ionic current assay that measures I_{Kr} or the current through an expressed I_{Kr} channel protein, such as that encoded by hERG (see sections 3.1.2 and 3.2.1)
3. Results from a ventricular repolarization assay that measures action potential parameters in isolated cardiac preparations (see sections 3.1.2 and 3.2.2) or specific electrophysiological parameters indicative of action potential duration in anesthetized animals (see sections 3.1.3 and 3.4.3)
4. Results from an *in vivo* QT assessment (see sections 3.1.3 and 3.4), either standard (see ICH S7A) or enhanced (see below)

Further description of figure

A positive signal from the pharmacological/chemical class is generated when the test substance belongs to a group of pharmaceuticals of which many, though not necessarily all, members have been shown to induce QT interval prolongation in humans.

Class can be defined by:

- therapeutic group (e.g., antipsychotics),
- mode of action (e.g., H-1 antihistamines, antiarrhythmics),
- chemical structure (e.g., fluoroquinolones).

A standard *in vivo* QT assessment should be a component of the safety pharmacology core battery cardiovascular study as described in ICH S7A. A rat or mouse study is generally not considered acceptable for assessing potential risk for QT interval prolongation in humans.

For an enhanced *in vivo* QT assessment, a variety of study designs can be appropriate and examples are provided in section 3.4. An enhanced *in vivo* QT assessment can be conducted as part of the core battery cardiovascular study, provided other cardiovascular parameters are collected in this or another nonclinical study. A rationale for conducting an enhanced *in vivo* QT assessment as the core battery cardiovascular study is to further evaluate concerns or signals in the assessments numbered 1-3 above and to avoid unnecessary use of animals (i.e., avoid performing both standard and enhanced QT assessments).

Follow-up studies are intended to provide greater depth of understanding or additional knowledge regarding observed effects (see ICH S7A, section 2.8 for further details). Information from follow-up studies could be related to potency, slope of the dose response curve, or magnitude of the nonclinical response. Another application is to determine whether an apparent positive or equivocal signal in an assay is the result of an artifact. Follow-up studies are designed to address specific issues, and, as a result, various *in vivo* or *in vitro* study designs can be applicable.

The design of enhanced and follow-up studies *in vivo* should reflect all available clinical and nonclinical information. Considerations for the design of these studies include:

- utility of repeated administration of test substance,
- use of appropriate positive control substances and reference compounds,
- selection of animal species and gender,
- measurement of electrophysiological parameters at multiple time points (including at T_{max}),
- information on metabolism, including plasma levels of parent compound and metabolites (including human data if available) and use of metabolic inducers or inhibitors, as appropriate,
- information on tissue distribution,
- use of adequate numbers of animals,
- potential for confounding effects in conscious animals such as pharmaceutical-induced effects on heart rate or autonomic tone, or toxicities such as tremor, convulsion, or emesis.

The design of follow-up studies *in vitro* can focus on issues such as activity of metabolites or inhibition of other channels not previously evaluated.

2.3.2 Further Considerations for the Nonclinical Testing Strategy

1. While scientific rationale for the selection of test systems and study design should always be provided, it is especially important to provide detailed justification for the selection of the *in vivo* test system and study design used to assess the risk for QT interval prolongation.
2. If assessment of the pharmacological/chemical class yields a positive signal and there is a positive result in the *in vivo* assay, additional nonclinical testing is not necessary to support initial clinical studies. A similar test strategy applies if there is a positive response in either the ion channel or the repolarization assay that is confirmed *in vivo*.
3. If the *in vivo* QT assessment does not confirm an initial positive signal, the nonclinical assessments 1-3 in section 2.3.1 should be completed prior to initiating clinical studies. If a specialized electrophysiology study in anesthetized animals (e.g., MAP or ERP assay, see section 3.4.3) is considered appropriate to satisfy the recommended *in vivo* QT assessment, then the results from this study can be used for both the repolarization assay and *in vivo* QT assessment.
4. In circumstances where clinical studies have not confirmed a signal of potential risk for QT interval prolongation observed in nonclinical studies, retrospective evaluation or follow-up nonclinical studies may be appropriate to understand the basis for the discrepancy (e.g., determination of metabolic differences or existence of large margins of safety).
5. If postmarketing or clinical study data suggest a potential QT interval prolongation effect despite negative findings in the available nonclinical studies, follow-up nonclinical studies to address this discrepancy can be valuable.

2.3.3 Implications of Nonclinical Studies

Any non-antiarrhythmic pharmaceutical that blocks repolarizing ionic currents (or enhances depolarizing currents), increases the cardiac action potential duration, prolongs the QT interval, or elicits arrhythmic events in nonclinical studies should be considered to pose a risk to humans, especially if *in vivo* effects occur at concentrations that are low multiples of the anticipated therapeutic plasma concentrations. On the other hand, the absence of findings in nonclinical studies for QT interval prolongation is not considered to preclude a potential risk to humans.

Evidence for repolarization impairment in nonclinical studies should not, however, preclude development of the candidate pharmaceutical. Such findings should entail more rigorous clinical and possibly nonclinical evaluation of the potential effects on QT interval prolongation. However, in the presence of strong nonclinical signals of risk for ventricular arrhythmia such as torsade de pointes, it can be prudent to undertake a risk versus potential benefit analysis.

In the presence of a signal of potential risk, a purpose of *in vivo* QT assessments is to provide nonclinical data to estimate margins of safety and guide clinical study design. However, even large margins of safety based on nonclinical data are not considered to be a basis for dismissing a signal of potential risk.

3. TEST SYSTEMS

3.1 General Considerations for Test Systems

This section provides an overview of methodologies currently used to assess the potential for pharmaceuticals to delay ventricular repolarization and to assess the potential risk for QT interval prolongation. The following criteria should be considered in selecting the most appropriate test systems for the candidate pharmaceutical:

- Assay methodology and experimental endpoints are well accepted by the scientific community as scientifically valid and robust.
- Assays and preparations can be standardized so that results can be accurately compared among laboratories.
- Endpoints/parameters of the assays are linked to human risk in a way that can be assessed in clinical trials.

3.1.1 Use of Positive Control Substances and Reference Compounds

Positive control substances should be used to establish the sensitivity of *in vitro* preparations for ionic current or action potential duration assay. In the case of *in vivo* studies, positive control substances should be used to validate and define the sensitivity of the test system, but need not be included in every experiment.

For test substances belonging to a pharmacological/chemical class that is associated with QT interval prolongation in humans, the use of concurrent reference compounds in *in vitro* and *in vivo* studies should be considered to facilitate ranking the potency of the test substance in relation to its comparators.

The use of reference compounds can also be valuable in assays designed to address positive or equivocal results from prior studies. If no reference compound is available from the pharmacological/chemical class represented by the test substance, the comparator should be selected on the basis of its ability to produce a relevant response in the assay and provide a useful context for the results observed with the test substance (see also ICH S7A).

Whether or not positive control substances or reference compounds are used in experiments should be justified.

3.1.2 In Vitro Electrophysiology Studies

In vitro electrophysiology studies can provide valuable information concerning the effect of a test substance on action potential duration and/or cardiac ionic currents. These assays have an important role in assessing the hazard for QT interval prolongation and elucidating cellular mechanisms affecting repolarization. *In vitro* electrophysiology studies employ either single cell (e.g., heterologous expression systems, disaggregated cardiomyocytes) or multicellular (e.g., Purkinje fiber, papillary muscle, trabeculae, perfused myocardium, isolated, intact heart) preparations. Multicellular preparations are stable model systems in which to study action potential duration. While more fragile, single cell preparations minimize diffusional barriers to the site of action. Heterologous expression systems, where human ion channel protein(s) are expressed in noncardiac cell lines, are used to assess the effects of a test substance on a specific ion channel. Disaggregated myocytes are technically more challenging than the expression systems but have the advantage of being suitable for assessing effects on both action potential duration and ionic currents.

Tissue and cell preparations for *in vitro* assays are obtained from laboratory animal species including rabbit, ferret, guinea pig, dog, swine, and occasionally human. Species differences in terms of which cardiac ion channels contribute to cardiac repolarization and to the duration of the action potential should be considered in selecting a test system. When native cardiac tissues or cells are used, the characteristics and source of the preparation should be considered because the distribution of ion channel types varies according to the region and type of cell.

Test substance concentrations for *in vitro* studies should span a broad range, covering and exceeding the anticipated maximal therapeutic plasma concentration. Ascending concentrations should be tested until a concentration-response curve has been characterized or physicochemical effects become concentration-limiting. Ideally, the duration of exposure should be sufficient to obtain steady-state electrophysiological effects, unless precluded by the viability of the cell or tissue preparation. The duration of exposure should be indicated. Appropriate positive control substances should be used to establish the sensitivity of the *in vitro* assay system as well as to confirm that the ion channels of interest are present and stable.

Problems that can confound or limit the interpretation of *in vitro* electrophysiology studies include the following:

- The testing of high concentrations of the test substance can be precluded by limited solubility in aqueous physiological salt solutions,
- Adsorption to glass or plastic or non-specific binding to the test matrix can reduce the concentration of the test substance in the incubation or perfusion medium. Measurement of concentration is important if safety margins are to be derived from these data,
- Test substance concentrations can be limited by cytotoxic or physicochemical attributes of the test substance that disrupt cell membrane integrity so that electrophysiological endpoints cannot be obtained,
- Because cardiac cells and tissues have limited capacity for drug metabolism, *in vitro* studies using the parent substance usually do not provide information on the potential effects of metabolites. When *in vivo* animal or clinical studies reveal QT interval prolongation that is not corroborated by *in vitro* studies using the parent substance, testing major metabolites in the *in vitro* test systems should be considered.
- Results from *in vitro* assays have an important role in hazard identification but alone are not considered reliable for predicting safety margins.

3.1.3 *In Vivo Electrophysiology Studies*

Intact animal models allow investigation of ventricular repolarization or associated arrhythmias where integrated effects on the full complement of ion channel and cell types are assessed. Also, potential neuronal and hormonal influences on the pharmacodynamic effect of the pharmaceuticals are present in animals.

The QT interval of the ECG is the most commonly used endpoint to gauge effects of a test substance on ventricular repolarization, which a variety of test systems can be used to monitor. In specialized electrophysiology studies, regional information regarding the ventricular repolarization (e.g., monophasic action potential duration and effective refractory period) can also be obtained from *in vivo* models. Additional

safety parameters of interest, including blood pressure, heart rate, PR interval, QRS duration, the presence of U waves, and arrhythmias, can be assessed simultaneously.

Laboratory species used for *in vivo* electrophysiology studies include dog, monkey, swine, rabbit, ferret, and guinea pig. The ionic mechanisms of repolarization in adult rats and mice differ from larger species, including humans (the primary ion currents controlling repolarization in adult rats and mice is I_{to}); therefore, use of these species is not considered appropriate. Each test system and test species has different utilities and no single system can comprehensively provide information on all parameters. The most appropriate *in vivo* test systems and species should be selected and justified.

The dose range should be in accord with that discussed in ICH S7A and, whenever feasible, should include and exceed the anticipated human exposure. The dose range can be limited by animal intolerance to the test substance, e.g., emesis, tremor, or hyperactivity. For studies designed to relate the extent of delayed ventricular repolarization to concentrations of the parent test substance and its metabolites, controlled exposure via constant intravenous infusion can be used. Monitoring exposure to the test substance and metabolites (see ICH S3A) provides opportunities to interpret dose- and concentration-response data and to design follow-up studies, if appropriate.

Factors that should be considered in conducting studies and interpreting the results are as follows:

- Data acquisition and analysis methods,
- Sensitivity and reproducibility of the test systems,
- Dosing period and measurement points,
- Heart rate and other cardiovascular effects that confound interpretation of QT interval data,
- Inter-species and gender differences, e.g., cardiac electrophysiology, hemodynamics, or metabolism of pharmaceuticals,
- Pharmaceuticals that have effects on several ion channels can yield complex dose-response relationships that could be difficult to interpret.

3.2 In Vitro Test Systems

3.2.1 In Vitro Ionic Current Assays

Most pharmaceuticals associated with torsade de pointes inhibit the rapidly-activating delayed rectifier current, I_{Kr} . Therefore particular attention to assays for I_{Kr} is prudent for assessing risk of QT interval prolongation. Inhibition of other outward (repolarizing) ionic currents (e.g., I_{to} , I_{K1} , and I_{Ks}) or increases in inward (depolarizing) ionic currents (e.g., I_{Na}) could also lead to QT interval prolongation and, therefore, should be considered when investigating the mechanism(s) for QT interval prolongation.

Using the voltage clamp technique, outward or inward ionic currents can be measured from various single cell preparations. Because of inherent difficulties associated with recording I_{Kr} in native myocytes, much of the available data for this current has been obtained using recombinant cell lines expressing hERG. Hazard identification and mechanism of action studies (e.g., voltage-dependence, state-dependence of block, and

channel kinetics) are conveniently performed in heterologous expression systems because there are fewer types of endogenous currents present. The relative potency of ionic current inhibition is most often expressed as the 50% inhibitory concentration (IC₅₀). The slow decrease in current that is often observed during whole cell voltage clamp (i.e., “rundown”) can make it difficult to accurately measure IC₅₀ values. It should also be noted that some pharmaceuticals show time-, voltage- or channel state-dependent interactions with channels that can influence the determination of IC₅₀ values.

3.2.1.1 Ionic Current Assays (I_{Kr} -like Currents) Using Heterologous Expression Systems and Cell Lines

When transfected with hERG alone or hERG in association with genes for potential regulating subunits (e.g., MiRP1), appropriate cell systems express a K⁺ channel that displays biophysical and pharmacological properties similar to I_{Kr}. Several expression systems have been used to test the activity of test substances on hERG current. Microinjection of hERG cRNA into *Xenopus* oocytes is a well-established method for expression of this and other channels. However, a major limitation of this model is that test substances can accumulate in the oocyte yolk, resulting in significant variability and error in potency estimates, even when compared to a positive control substance. Stable, mammalian cell lines expressing hERG are preferred because they do not share this limitation. So far, most studies have used Chinese hamster ovary cells (CHO), mouse fibroblasts (Ltk- cells), and human embryonic kidney cells (HEK293).

The cell line should be selected based upon appropriate levels of hERG expression. The sensitivity of expressed hERG should be tested using standard hERG blockers (e.g., E4031, dofetilide) as positive controls on the same batch of cells used for safety testing. Also, the cell line should be tested at appropriate intervals to confirm the stability of the current from the expressed channel. The presence of endogenous currents, the presence or absence of subunits (directly associated regulatory proteins), and kinases or phosphatases controlling regulatory phosphorylation sites can affect the pharmacology of the expressed channel protein relative to native cardiac ion channels.

Mammalian cell lines stably expressing hERG (HEK 293, CHO, Ltk-, etc.)

hERG cDNA is transfected into a suitable heterologous expression system. The current mediated through the expressed channel protein is measured using standard voltage clamp techniques. Studies comparing different test substances should be conducted under the same experimental conditions. While potencies of hERG blocking substances are generally similar in all mammalian cell lines evaluated and are consistent with potencies for blocking native I_{Kr} in ventricular myocytes, it should be noted that hERG encodes only the α -subunit of human I_{Kr} channel. It is possible that a test substance might block I_{Kr} via interacting directly with a β -subunit; such a mechanism will not be detected in these hERG expression assays.

References:

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AT-1 cells and HL-1 cell line

Atrial tumor cells derived from transgenic mouse myocytes (AT-1 cells) and the immortalized cardiac muscle cell line (HL-1) have been employed as test systems containing I_{Kr} . AT-1 cells cannot be passaged in culture and, since they are dividing cells, their phenotype is abnormal compared to differentiated adult myocytes. HL-1 cells are derived from AT-1 cells and can be passaged but, like AT-1 cells, are dividing cells and less differentiated than adult myocytes. Each cell type can be studied using standard voltage clamp methodology. The phenotype of these cells allows a determination of effects on many of the major cardiac ion channels (sodium, calcium, and potassium (ERG)). The channels expressed are similar to neonatal/fetal mouse cardiac myocytes raising some concerns about de-differentiation in these tumor-derived cells. For example, I_{Kr} is not prominent in adult mouse heart, but is present in fetal heart. In addition, the tumor cells are of atrial, not ventricular, origin and there is limited pharmacological characterization of the currents. Nevertheless, these cells may prove more useful with better characterization of the model.

References:

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Yang T, Snyders D, Roden D. Drug block of I_{Kr} : Model systems and relevance to human arrhythmias. *J Cardiovasc Pharmacol* **38**:737-744, 2001.

3.2.1.2 Ionic Current Assays Using Disaggregated Cardiac Myocytes

Use of ventricular myocytes from animals or humans to study effects on cardiac ionic currents obviates concerns about channel composition and regulatory components because the channel is studied in its native cell. Isolated myocyte preparations are advantageous in that multiple myocytes can be obtained from one heart, subsequent studies can identify the ionic mechanisms responsible for repolarization changes using the same preparation, and diffusional barriers are minimal. While isolated myocytes can be obtained from human cardiac specimens, availability is usually limited to atria or diseased ventricle and preparations can be compromised. Isolated myocytes (e.g., from dog, rabbit, guinea pig) should possess functional I_{Kr} as demonstrated using positive control substances (e.g., dofetilide, E-4031).

Enzymatic treatments used in the disaggregation procedure can alter channel function, leading to potentially greater variability of results, “loss” of currents, and difficulties in standardizing data across laboratories. Disaggregated myocytes also exhibit a higher variability of action potential duration (even when paced at a constant stimulation rate), likely due to the absence of electrical coupling present in syncytial preparations.

Conventional whole cell patch clamp recording of ionic currents in myocytes

Multiple ionic currents with overlapping kinetics and pharmacology, and variable expression of channels in different species complicate the interpretation of studies in native preparations. Separation of repolarizing from depolarizing currents requires inhibition of Ca^{2+} current by selective blockers (e.g., nitrendipine) and suppression of Na^{+} current by depolarizing holding potentials (e.g., -40 mV) or use of blockers (e.g., tetrodotoxin). Selective potassium channel blockers (e.g., E-4031 to block I_{Kr}) can be used to isolate I_{Kr} and I_{Ks} repolarizing components.

References:

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3.2.2 In Vitro Action Potential Duration Assays

Action potential recordings reflect the integrated activities of the multiple ion channels, pumps, and exchanger mechanisms that contribute to the depolarization phase and repolarization process at the cellular level. Therefore, assessing changes in action potential duration can be a comprehensive approach for identifying effects of a test substance on repolarization. When a positive effect on one of the action potential parameters is observed in an *in vitro* assay, ionic current studies should be conducted to elucidate the cellular mechanism for the effect. Action potential duration can be measured in multicellular preparations and isolated ventricular myocytes but can also be evaluated in *in vivo* models (see section 3.4.3).

Parameters that provide useful information for QT interval prolongation and related proarrhythmic potential of a test substance include action potential duration (APD) at specific degrees of repolarization, e.g., action potential duration at 90% repolarization (APD₉₀). Changes in other action potential parameters, including resting membrane potential, action potential amplitude, and maximum rate of depolarization (V_{max}), can also be useful in assessing the electrophysiological action of a test substance on the heart. Important indicators of proarrhythmia, such as early afterdepolarizations and triggered activity can also be observed in this preparation.

Most blockers of I_{Kr} induce greater action potential prolongation at slower versus faster stimulation rates (reverse use-dependency). Thus, it is recommended to use more than one stimulation rate at multiple test substance concentrations before excluding the possibility of an effect. Exploration of a broad range of test substance concentrations is important, as a bell-shaped concentration-response curve can be obtained if the selectivity of the test substance for a particular ion channel becomes generalized to another ion channel at higher concentrations.

Concentration-dependent prolongation of the action potential duration *in vitro* and reverse rate-dependent effects should be considered as potential signals of QT interval prolongation in clinical settings. Because not all pharmaceuticals that prolong the QT interval in humans increase the action potential duration of all tissues and species, action potential studies should be considered along with *in vitro* ionic current studies (e.g., hERG) and *in vivo* animal studies before dismissing the possibility of a positive effect.

References:

Cavero I, Mestre M, Guillon JM, Crumb W: Drug that prolong QT interval as an unwanted effect: assessing their likelihood of inducing hazardous cardiac dysrhythmias. *Exp Opin Pharmacother*, **1**: 947-973, 2000.

De Ponti F, Poluzzi E, Montanaro N. QT-interval prolongation by non-cardiac drugs: lessons to be learned from recent experience. *Eur J Pharmacol*; **56**: 1-18, 2000.

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

3.2.2.1 Action Potential Duration Studies Using Disaggregated Cardiac Myocytes

Myocytes are isolated using collagenase solutions and the action potential of a single cell is measured by impaling a cell with a fine-tipped electrode and recording the voltage difference across the cell membrane. In contrast to voltage clamp studies with this preparation (see 3.2.1.2), the cell is not dialyzed and there is less rundown of currents. With careful selection of experimental conditions, both action potential and ionic current measurements can be accomplished in the same cell or cell type. This is a powerful approach to elucidate the ionic current mechanism responsible for a change in action potential parameters. Compared to action potential recordings in multicellular preparations, the lack of gap junctions in the single cell leads to increased beat-to-beat variability in action potential duration and tends to exaggerate regional differences in action potential duration. Like other single cell assays, results from these assays are considered more valuable for mechanism of action and hazard identification than for establishing safety margins.

Reference:

Salata JJ, Jurkiewicz NK, Wallace AA, Stupienski RF III, Guinosso PJ Jr, Lynch JJ Jr. Cardiac electrophysiological actions of the histamine H1-receptor antagonists astemizole and terfenadine compared with chlorpheniramine and pyrilamine. *Circ Res* **76**:110-119, 1995.

3.2.2.2 Action Potential Duration in Multicellular Preparations

Excised, multicellular tissue preparations are employed in *in vitro* cardiac action potential studies. In these protocols, a fine-tipped electrode impales a single myocyte on or near the exposed surface of the preparation and the voltage difference across the cell membrane is recorded. Modifications of the action potential shape and parameters by a test substance will reflect potential pharmacological effects on native channels (including accessory subunits, natural channel environments, and second messenger systems) as well as potential influences of communication among cells. The experimental conditions (including stimulation rate, test substance concentrations, and extracellular ion concentrations) can be manipulated in an attempt to mimic predisposing factors for QT interval prolongation and torsade de pointes. Perfusion to inner tissue segments of the multicellular preparation can be limited for some test substances. Metabolites can be tested in separate series of experiments. These assays are useful for hazard identification, but further investigation should be done in order to establish safety margins.

Action potential recordings in Purkinje fibers, papillary muscle, ventricular trabeculae

Following removal from the donor animal, the tissue is placed in an organ bath where it is superfused with physiological salt solution at a fixed temperature. Action potentials are recorded by impaling a cell on the surface of the tissue with a fine-tipped glass microelectrode. Prior to administration of a test substance, the excised tissue should undergo a period of stabilization to allow recovery from any electrophysiological instability related to dissection trauma. Also, the condition of the tissue preparation should be assessed for absence of spontaneous activity or experimental artifacts (e.g., due to hypoxia, movement of microelectrode, or damage to tissue during dissection). Tissue exposure to the test substance is achieved by dissolving the test substance in the incubation medium. Solvent/vehicle should be tested separately.

References:

Adamantidis MM, Lacroix DL, Caron JF et al. Electrophysiological and arrhythmogenic effects of the histamine type-1 receptor antagonist astemizole on rabbit Purkinje fibres: clinical relevance. *J Cardiovas Pharmacol.* **26**:319-327, 1995

Dumotier B, Adamantidis M, Puisieux F, Bastide M, Dupuis B. Repercussions of pharmacologic reduction in ionic currents on action potential configuration in rabbit Purkinje fibers: are they indicative of proarrhythmic potential ? *Drug Dev Res* **47**: 63-76, 1999.

Puisieux F, Adamantidis M, Dumotier B, Dupuis B. Cisapride-induced prolongation of cardiac action potential and early afterdepolarizations in rabbit Purkinje fibres. *Br J Pharmacol* **117**: 1377-1379, 1996.

Action potential recordings in preparations from ventricular muscle

Action potentials can be recorded from different layers across the ventricular wall using wedges or thinly sliced ventricular muscle. The cells from the endocardium to epicardium display different densities of channel subtype populations and therefore could respond differently to test substances. An increase in heterogeneity in action potential prolongation across the ventricular wall by a test substance has been proposed as a proarrhythmic mechanism. However, it is not known if the extent of heterogeneity modifications is exaggerated in these preparations compared to *in vivo*. These preparations are technically difficult and linked with a small database at present. More data would allow a consensus on the scientific value of this approach.

References:

Gan-Xin Y, Shimizu W, Antzelevitch C. Characteristics and Distribution of M Cells in Arterially Perfused Canine Left Ventricular Wedge Preparations. *Circulation* **98**:1921-1927, 1998.

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Drouin E, Charpentier F, Gauthier C, Laurent K, Le Marec H. Electrophysiologic characteristics of cells spanning the left ventricular wall of human heart: Evidence for presence of M cells. *J Am Coll Cardiol* **26**: 185-192, 1995.

Action potential recordings in isolated intact heart (Langendorff heart preparation)

Action potential recordings (either transmembrane or monophasic action potential recordings from cells on the surface of the myocardium) and ECG recordings can be obtained from isolated hearts where the coronary circulation is perfused retrogradely with physiological salt solutions or blood (commonly known as Langendorff heart preparation). Rabbit and guinea pig are commonly used. The heart can beat spontaneously or be paced at a fixed rate. Different concentrations of test substances are added to the coronary perfusion solution or blood. Since the preparation is perfused via its native vasculature, perfusion barriers to test substances are reduced compared to other multicellular preparations. Ventricular arrhythmias can be elicited with test substances that delay repolarization. This preparation represents a higher level of integration than single cell or excised tissue preparations but it is technically challenging to achieve a stable preparation and electrophysiological parameters. The heart becomes edematous via perfusion with physiological salt solution, which limits the duration of the experiment. Impalements with floating electrodes for action potential recordings are difficult to maintain in the contracting heart. Strict acceptance criteria should be established to reduce the likelihood of using hearts with sub-optimal viability.

References:

Benardeau, et al., J Pharmacol Exp Ther **292**:561-575, 2000.

Eckard L, Haverkamp W, Borggreffe M, Breithard G. Drug-related Torsades de Pointes in the isolated rabbit heart: comparison of clofilium, d,l-sotalol, and erythromycin. J Cardiovasc Pharmacol **32**: 425-434, 1998.

Hondeghem LM, Carlsson L, Duker G. Instability and triangulation of the action potential predict serious proarrhythmia, but action potential duration prolongation is antiarrhythmic. Circulation **103**:2004-13, 2001.

3.3 High Throughput *In Vitro* Screening Assays

High throughput potassium channel assays are being developed that utilize biophysical measures of membrane potential. In one example, fluorescent dyes that respond to alterations in transmembrane potential are used. Cultured cells transfected with hERG (or other ion channel cDNA such as KvLQT1 and MinK to test for inhibition of I_{Ks}) are engineered in a configuration such that changes in the intensity of their fluorescent signal reflect inhibition of the current. This is a promising approach that will enable drug discovery teams to screen chemical leads early in a program for unwanted ion channel activity such as hERG blockade. While these novel ion channel activity assays can be useful in preliminary screening of test substances to identify lead candidates for further electrophysiological testing, more experience would establish whether they have sufficient predictive value as an alternative to voltage clamp assays.

Another screening approach is use of competition binding protocols in which test substances are studied for their ability to displace a radiolabeled hERG channel blocker, such as dofetilide, from a cell line expressing hERG. However, competition for radioligand-binding sites provides no information on agonistic or antagonistic effects of the test substance on I_{Kr} . Moreover, this assay will not identify test substances that bind to hERG at sites other than the radioligand binding sites. Based upon these potential limitations, this assay is not considered a substitute for voltage clamp assays described above.

Reference:

Netzer R, Ebneith A, Bischoff U, Pongs O. Screening lead compounds for QT interval prolongation. Drug Discovery Today **6**, (2) 2001

3.4 *In Vivo* Test Systems

3.4.1 *Factors That Should Be Considered in Conducting Studies and Interpreting Results*

3.4.1.1 *Acquisition and Analysis of the QT Interval*

Although multiple lead ECG recordings might be desirable, ECG recordings from a single lead configuration with high quality and fidelity to detect a well-defined and reproducible T wave are considered acceptable to measure the QT interval, assuming other ECG parameters can be monitored from this lead. Typically, chest leads are considered preferable to limb leads since they yield more distinct and reproducible T waves. Recent advancements in technology, such as automatic analysis of consecutive beats over several minutes, facilitate the acquisition of QT interval data. If the end of

the T wave is difficult to detect by computerized ECG analysis software, the QT interval should be read manually. In all cases, the investigator should visually confirm the accuracy of a computer-measured QT interval.

QT intervals in the presence of the test substance should be compared to both baseline and concurrent vehicle control values. Changes in QT interval from baseline should be provided. It is considered inappropriate to compare QT interval on test substances to historical values since only extremely large QT effects are discernible by this methodology.

3.4.1.2 Sensitivity and Reproducibility of In Vivo Test Systems

Many factors influence the sensitivity and reproducibility of *in vivo* test systems. Conscious animals are often used in assessing the effect of test substances on the QT interval and, according to ICH S7A, are the preferred model for safety pharmacology assessment. However, in some cases, it can be more difficult to detect treatment-induced changes in conscious animals than in anesthetized animals because of large variability in the heart rate, movement, or other factors. In this case, anesthetized animals could be used for an enhanced *in vivo* QT assessment (see section 3.4.3).

For the reasons given above, the sensitivity and reproducibility of the *in vivo* test system should be characterized. This is particularly important to support conclusions that a test substance is without effect on the QT interval. For the appropriate use of positive and negative controls, see section 3.1.1.

3.4.1.3 Dosing Period and Measurement Points

Electrocardiographic parameters should be monitored prior to dosing and, when possible, throughout the full duration of the pharmacodynamic effect of the test substance, with particular efforts to capture the effect at T_{max} and bracketing T_{max} . Measurements at multiple time points, which can be incorporated into an enhanced study design, can improve the detection of effects related to the test substance.

It is possible that the effects of some test substances on the QT interval are more apparent after repeated dose administration. Although the mechanisms are not fully understood, this might be caused by accumulation of the test substance (or its metabolites) in cardiac tissue or by other mechanisms such as down-regulation of the ion channels. Therefore, steady state effects of the test substances on the QT interval should be evaluated following multiple dose administration, if concerns remain following analysis of standard *in vivo* QT assessment, or if pharmacokinetic data suggest that the test substance can accumulate in cardiac tissue.

3.4.1.4 Influence of Heart Rate Change on the QT Interval

The QT interval and heart rate have an inverse, non-linear relationship, which can vary among species, between animals, or even within the same animal at different heart rates. Thus, a change in heart rate exerts an effect on QT interval, which can confound the assessment of the effect of the test substance on the QT interval. There are two situations where there is variability in heart rate among animals: one where there is variability due to difference in autonomic tone, and the other where there is variability due to effects of test substances on heart rate. Therefore, the interpretation of data from *in vivo* test systems should take into account the effect of coincident changes in heart rate. Ideally, QT interval data obtained after administration of a test substance should be compared with control and baseline data at similar heart rates. When this is not feasible, there are several approaches to

address this problem. When the variability is not due to the test substance, it can be reduced by training or the use of anesthetized animal models. When the effects are due to test substances, other approaches should be used. The most common is use of a correction formula such as Bazett or Fridericia; however, these corrections can yield misleading data, especially when differences in heart rate between treatment and control are large. Analyses of QT intervals over a wide range of heart rates can provide more detailed information and increased predictability of the potential effect of a test substance. An alternative approach is to maintain a constant heart rate using cardiac pacing (see section 3.4.3).

3.4.1.5 Test Substances with Effects on Multiple Ion Channels

Test substances that have effects on more than one ion channel can yield complex dose-response relationships for QT effects. Dose-response relationships for other ECG and cardiac parameters can be helpful in interpreting complex dose-response relationships. *In vitro* studies can also help interpret complex *in vivo* findings.

3.4.2 Conscious Animals

The use of conscious animals offers numerous advantages. With regard to administration route, either oral or parenteral dosing is usually possible, and both single and repeat dose studies can be performed. Conscious animals can be used in studies of Latin Square crossover design, which have the advantage of greater statistical power for a given number of subjects. Alternatively, with a premise that there is no carryover effect, an escalating dose design is useful to find the minimum dose (concentration) of the test substance that affects ventricular repolarization. An additional advantage of conscious animals is their suitability for re-use in subsequent studies of other test substances, following an adequate washout period and verification of normal biochemical and functional parameters. A key to obtaining high quality and interpretable ECG data is to ensure that animals are acclimated to the laboratory environment and handling procedures, as this will minimize variations in cardiovascular parameters (most often tachycardia).

3.4.2.1 ECG Recordings in Unrestrained Animals

Unrestrained animals are minimally stressed, and the data can be obtained under physiological conditions. Because normal activity (e.g., eating, exercising, and sleeping) can produce large changes in heart rate, and movement can influence ECG waveforms, data to be analyzed should be carefully selected and standardized.

Telemetry

In telemetry test systems, data are collected from animals implanted with radiotransmitters. Data can be captured for extended periods of time following either single or multiple dose administration of test substance. Commonly used endpoints, in addition to ECG recordings, include blood pressure, heart rate, and behavioral activity. Technology is currently available for telemetric instrumentation of dogs, pigs, rabbits, guinea pigs, and monkeys. These systems call for an appropriate level of surgical sophistication and maintenance of model.

Holter

Holter ECG is an alternative data collection method for evaluating ECG changes in conscious animals. Unlike telemetry systems, Holter ECG methodology does not involve a surgical procedure, although animals should be trained to wear a jacket that holds the recorder. The Holter methodology provides continuous

recording of the ECG from multiple leads for several days. The duration of Holter experiments is limited compared to telemetry because the Holter ECG electrodes can irritate the skin of the animals. Because Holter ECG recording is hard-wired, the signal-to-noise ratio and the quality of the ECG are usually higher than that available with telemetry. Because of the weight and size of the recorder, Holter ECG recording is limited to larger animals such as dogs and monkeys.

3.4.2.2 ECG Recordings in Restrained Animals

ECG recordings and other data can be collected from animals acclimated to restraint procedures (e.g., sling for dogs and chair for monkeys) or trained to maintain a fixed position (e.g., laterally recumbent for dogs). Telemetry, Holter, or conventional ECG (acute placement of ECG leads) methodology can be used to capture these data. Advantages of this approach compared to one using unrestrained animals include stability of the ECG waveform (because of less animal movement), ease of oral and parenteral administration, including intravenous infusions, and ease of sampling blood for pharmacokinetic parameters. The duration of data collection is limited to the animal's tolerance to the restraint procedure. Animals can be surgically instrumented for evaluating cardiovascular hemodynamic parameters in addition to ECG recordings.

3.4.3 Anesthetized Animals

ECG data can be recorded in anesthetized animals, which allows for a stable preparation. Consideration should be given to the choice of anesthetic and how it can influence electrophysiological parameters. Monitoring hemodynamics (e.g., blood pressure), respiratory conditions (e.g., hemoglobin oxygen saturation with a pulse-oxymeter), metabolic status (e.g., acid-base balance), body temperature and/or plasma electrolytes are recommended. Beside QT interval, regional measures of repolarization (e.g., monophasic action potential duration and effective refractory periods) can be monitored. These can be readily correlated with exposure due to the ease of collecting plasma samples. Routes of administration of test substances are limited to parenteral and intraduodenal, and the study duration is limited to several hours. Use of anesthetized animals is generally not considered appropriate to achieve blood pressure and heart rate objectives of the core cardiovascular ICH S7A assessment.

Specialized Electrophysiology In Anesthetized Animals

The objectives of specialized electrophysiology studies are to obtain more direct measures of ventricular repolarization while attempting to minimize confounding experimental effects (e.g., heart rate changes). Many of these study designs can be appropriate for enhanced or follow-up *in vivo* QT assessments since these test systems are used to further characterize the effects of a test substance on ventricular repolarization.

Heart rate control

Maintaining the heart rate by cardiac pacing is a useful approach for assessing the net effect of test substances on ventricular repolarization, avoiding the influence of heart rate variation. Cardiac pacing can be achieved by use of either an intracardiac pacing catheter or epicardial pacing electrodes in combination with a cardiac stimulator. The pacing stimulation frequency will be faster than the original ventricular rhythm to achieve overdrive suppression and

should account for any effects of the test substance on heart rate. I_{Kr} blockers generally demonstrate reverse use-dependency; therefore, the use of animals with slower heart rates can optimize the detection of delayed ventricular repolarization by this class of test substances. However, the benefit of heart rate stability provided by pacing can outweigh the disadvantage of having to evaluate the effect of test substances on the QT interval at moderately elevated heart rates. Alternatively, slower heart rates can be achieved in pacing studies when atrioventricular blockade is used.

Ventricular monophasic action potential (MAP)

Monophasic action potential recordings can be used to assess repolarization (see section 2.3.1) and provide a bridge between cellular and *in vivo* electrophysiology. Since MAP duration measurement reflects the repolarization duration at one restricted region, it is recommended that MAP recordings be obtained simultaneously with surface ECG recordings. A benefit of MAP recordings is that the waveform of MAP is simple, and its duration (e.g., MAPD₉₀) can be measured accurately with a computer. In *in vivo* preparations, epicardial and endocardial MAP can be measured using a hand-held contact electrode probe and a contact electrode catheter, respectively. MAP can also be recorded in *in vitro* Langendorff-perfused hearts of smaller animals, such as rabbits, as indicated in section 3.2.2.2.

When compared with *in vitro* intracellular recordings of action potentials, MAP measurement has the advantage of allowing *in vivo* recordings from the intact heart. Because MAP is not a single-cell recording, the averaged electrophysiological properties over many cells make recordings more representative. However, as a potential disadvantage, distinct features of individual myocardial cells might be masked. No reliable information on maximal upstroke velocity can be obtained. Therefore, it is not considered possible to evaluate the effects of test substances on the cardiac Na⁺ channel using this parameter.

Ventricular effective refractory period (ERP)

Myocardial cells are refractory to an extrastimulus during the depolarized state, and regain excitability during the repolarization phase. Ventricular ERP is the period of refractoriness of the ventricle at a certain condition (cycle length and stimulation voltage are fixed) and, in combination with ECG data from the same animals, can be used to address the assessment of repolarization (see section 2.3.1). This parameter can be measured using programmed electrical stimulation. Electrical stimulation threshold should be monitored because it affects the duration of ERP. Measurement of QT interval should also be conducted in this study. Usually MAP duration and the ERP are correlated and either can be used for assessment of effects on repolarization.

3.4.4 Simulated Pathological Conditions and Arrhythmias

The precise relationship between pharmaceutical-induced delay of ventricular repolarization and risk of proarrhythmia is not known. Directly assessing the proarrhythmic risk of pharmaceuticals that prolong the QT interval would be a logical undertaking; however, modeling of the clinical condition where pharmaceuticals elicit arrhythmia is complicated. Therefore, animal models of proarrhythmia are not presently recommended for routine evaluation, since these models are insufficiently established to provide any certainty of detecting relevant effects.