SAFETY PHARMACOLOGY ASSESSMENT AND ASSOCIATED REGULATIONS

Jega Iswaran
Biota Holdings Ltd, 10/585 Blackburn Road, Notting Hill, Vic 3168, Australia

Jorma Ahokas
School of Medical Sciences, RMIT-University, Bundoora, Victoria 3083, Australia

Keywords: safety pharmacology, non-clinical pharmacology assays, regulatory guidelines

Contents

1. Introduction
2. Drug screening studies for assessing pharmacological activity
3. Safety pharmacology studies in the context of regulatory guidelines
4. Use of *in vitro* systems and *in vivo* models for safety pharmacology testing
4.1. Core Battery Studies
4.1.1. Cardiovascular System
4.1.2. Respiratory System
4.1.3. Central Nervous System
4.2. Supplemental Studies
4.2.1. Renal/Urinary System
4.2.2. Gastro-intestinal System
4.2.3. Autonomic Nervous System
4.2.4. Immune System
4.2.5. Dependency Potential
4.2.6. Effects on Skeletal Muscle
4.2.7. Endocrine System
5. Concluding Remarks
Glossary
Bibliography
Biographical Sketches

Summary

The aim of safety pharmacology studies is to identify adverse effects of new molecular entities (NMEs) and biological products that are administered to humans. They are done to evaluate adverse pharmacodynamic and/or pathophysiological effects. The safety pharmacology testing of NMEs aims to identify effects in biological systems that are unrelated to the intended primary pharmacological target. The results from such investigations form the basis for selecting NMEs for clinical development or for recommending the clinical and laboratory indices that should be monitored in the early phase clinical trials conducted during a new product development program. A set of core studies has been identified by regulatory agencies for regular investigations with all NMEs. Depending on the need, a second tier or supplemental studies may also be conducted selectively either prior to first administration to human, or in parallel with

early phase human studies provided there is no immediate concern for safety.

This chapter details selected current methodologies and focuses on the major organ systems, including the cardiovascular, respiratory and nervous systems, as well as the endocrine and immune systems.

1. Introduction

The word pharmacology is derived from the two Greek words, *pharmacon* (medicine) and *logos* (study). Pharmacology is the scientific study of the effects of therapeutically useful chemical agents and the underlying mechanisms by which they exert their biological responses. The term pharmacodynamics is sometimes used interchangeably with pharmacology, but it refers more specifically to the study, at molecular level, of the biochemical and physiological effects of therapeutic agents on cellular systems. A wide range of techniques is used in characterizing the pharmacological action of a new molecular entity (NME). They include biochemical, molecular and cellular methodologies. For regulatory purposes and in pharmaceutical development, three categories of pharmacological research are generally recognized, namely primary pharmacodynamics, secondary pharmacodynamics and safety pharmacology. Primary pharmacodynamic studies relate to the establishment of all aspects of the desired therapeutic effect as demonstrated in experimental *in vitro* and *in vivo* studies, i.e. primary action in the target system. Secondary pharmacodynamic investigations address the resultant action in the target system and effects that are outside the realms of primary therapeutic activity. Safety pharmacology investigations refer to pharmacodynamic effects in non-target systems that lead to side effects.

Safety pharmacology has evolved as an integrated discipline from the distinct fields of pharmacology, physiology and toxicology. The origins of safety pharmacology are based on observations that, organ functions can be toxicological targets in humans exposed to novel therapeutic agents. However, the drug effects on organ functions (unlike organ structures) are not readily detected by standard toxicological testing.

2. Drug Screening Studies for Assessing Pharmacological Activity

The need for a rapid and cost-effective method of addressing potential liabilities of drugs at the early discovery stage of lead selection and lead optimization has been referred to as '*in vitro* safety pharmacology profiling' (*in vitro* SPP). It employs a large number of relatively inexpensive *in vitro* assays to do molecular target profiling of NMEs by *in vitro* SPP. By focusing on early hazard identification it can flag receptor-, enzyme-, transporter-, and channel-related liabilities. Interpretation of the data is aided by evaluation of results in conjunction with preliminary bioavailability and toxicity characteristics determined either *in vitro* or *in vivo.*

At the research stages and prior to administration to humans, NMEs are subjected to biological assays that explore molecular mechanisms of action and pharmacology.

The objective in these studies is to evaluate the potential for these compounds for use as therapeutic agents. A variety of testing methods can be used for this purpose and they

include: computer-assisted (*in-silico*) modeling, cloned receptors, cultured cells, isolated tissues or organs, specific enzyme inhibition or activation and animal models of human disease. They are intended to represent aspects of sub-molecular, molecular, cellular, organ or whole body components in a cascade of biological screening techniques. *In vitro* studies cover quantitative investigations at molecular and cellular levels providing for effective concentrations (EC_{50}) or inhibitory concentrations (IC_{50}) of an NME as an index of its potency. *In vivo* studies on the other hand, apply to in-life studies and are associated with animal experimentation. In applying these options, the use of validated assays and comparator (or reference) drugs will be of great benefit in reliably ranking the relative efficacy or activity.

In the design of studies, due consideration should be given to selection of sample size, use of controls and reference compounds. Analysis and interpretation of the results of pharmacodynamic activity are also usually considered in relation to the associated pharmacokinetics (drug disposition) and metabolism of the NME. If a potential exists for accumulation of a drug in plasma or tissues following repeated administration, safety pharmacology studies should consider a 7 to 14-day repeat dose protocol.

3. Safety Pharmacology Studies in the Context of Regulatory Guidelines

Whilst 'efficacy' studies in pharmacology deal with primary pharmacodynamic properties of a drug or NME, safety pharmacology studies are designed to investigate the potential undesirable pharmacodynamic effects of a substance on physiological functions in relation to exposure in the therapeutic range and above (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use [ICH] S7A Guidelines, 2000). The safety pharmacologist uses knowledge of physiology, biochemistry, anatomy, and cellular and molecular biology to evaluate and assess the safety profile of a new chemical or biological entity in a hierarchy of physiological systems necessary for survival. In these studies, functional indices of potential toxicity are measured (ICH S6 Guidelines, 1997). The doses selected for the studies may, therefore, have the potential to cause unwanted effects in non-target systems leading to side effects. In addition to the separately conducted toxicity assessments with new compounds, safety pharmacology studies provide important information to clinical investigators for potential acute adverse effects upon a number of body systems, such as cardiovascular, respiratory and central nervous system functions (Dixit, 2004). The ICH S7A Guidelines provide general information such as definitions, general principles, timing of studies, core battery of studies to be considered and compliance with Good Laboratory Practice. These concepts also apply to biotechnology-derived pharmaceuticals.

Cardiac side (adverse) effects of drugs, not intended for use in heart disease, have been recognized for sometime now and have become a main focus in selection of new products for pharmaceutical development. A complementary guideline has been developed that focuses on cardiac function effects of human pharmaceutical products and, more specifically, to assist in the evaluation of risk of ventricular repolarization-associated cardiac embarrassment (ICH S7B, 2005).

The afore-mentioned ICH guidelines have now been adopted by all major

pharmaceutical development and marketing territories of the world (USA, European Union and Japan) and are in use in the expectation that effects of an NME are investigated as part of the safety evaluation program prior to first administration to humans. This paper discusses the salient aspects of safety pharmacology studies that have been adopted by the pharmaceutical industry to address some of these concerns.

4. Use of *in vitro* Systems and *in vivo* Models for Safety Pharmacology Testing

Although the concept and importance of characterizing drug related non-target pharmacology that can lead to side effects has been recognized for a considerable period of time, it is the ICH initiative of the 1990s that has accelerated the thinking process and provided a consolidated position for the pharmaceutical industry to move forward on this front. A valuable compendium on aspects of safety pharmacology studies conducted at the present time, and a status update, have been published in the *Journal of Pharmacological and Toxicological Methods* (Pugsley, 2005). Another two publications relevant to this topic are the books by Gad (2004) and Vogel *et al.* (2006). They cover historical background information as well as providing a systemic approach to screening and designing safety pharmacology investigations, and also discuss the significance of integrating those endpoints into the wider context of drug disposition and pharmaceutical safety assessment.

Test systems, methods and standardized protocols for safety pharmacology studies are now on offer by Contract Research Organisations (CROs) at reasonable costs to a sponsor company screening NMEs. It would be up to a sponsor pharmaceutical company to decide exactly which tests are appropriate for the type of compound(s) in question. For *in vivo* studies, careful consideration should also be given to selection of animal species that would permit data comparison from other relevant studies within the program and the use of established methods and techniques that have been adequately validated in the laboratory conducting the studies. At least in part, these will help ensure quality standards and reproducibility of data and, therefore, a reliable extrapolation to the human situation. A further key consideration is that all safety pharmacology studies should be conducted under conditions of Good Laboratory Practice (GLP) and be officially certified as compliant with the regulations (US Food and Drug Administration [FDA], 1978; Organisation for Economic Cooperation and Development [OECD], 1998a; OECD,2002; OECD, 2004). GLP is now an important part of the quality management of pharmaceutical laboratory data generation to provide a higher level of confidence in the reproducibility of data. They are a reflection of sound study management that ensures reliability and integrity of studies. In conducting these tests, it is usual to select a range of dose or concentration levels from clinically relevant to doses that are high but not toxic.

4.1. Core Battery Studies

Since undesirable pharmacodynamic effects to drugs may be related to a specific action on any of the non-primary target tissues or organs, in an extreme situation, it may be argued that safety pharmacology investigations should be conducted on all systems in the body. This would lead to a lengthy and expensive process of testing a new NME or a biological product. However, logistical and economic considerations are likely to dictate

a more rational approach and, therefore, the regulatory guidelines do recommend that a hierarchy of organ systems be developed, such as those that govern vital functions critical to life.

This has led to a general acceptance of cardiovascular, respiratory and central nervous system assessments as the important systems to be investigated as a 'core battery' of studies.

It would be expected that additional studies for investigating potential for affecting other systems would be identified as appropriate on a case by case basis for a given compound, dependent upon its chemical, biological and/or known toxicological characteristics. In the event of excluding investigations into a core battery study or system, these need to be scientifically justified (ICH S7A, 2000).

Further refinement by way of a schedule is offered by Redfern *et al.,* (2002) in their analysis of safety pharmacology studies, which suggests a 'tiered approach' to conducting these studies. In this step-wise approach, the *in vitro* studies would be positioned in the early stages of investigations of an NME (see Table 1).

Lead Identification and Optimisation (>100 compounds) - 'In silico' assessment - 'Key' receptors - 'Key' enzymes - 'Key' ion channels
Candidate Drug Selection (2-4 compounds) - 'Receptogram' partial profiling - Functional observational battery (FOB) - *In vitro* cardiac electrophysiology - Dog telemetry
Candidate Drug Evaluation (pre-Phase 1) (1-2 compounds) - Complete 'receptogram' profiling - Haemodynamics - Respiratory Function - Gastro-intestinal function - Tremor - Motor co-ordination - Learning and memory - Auditory Function - Locomotor activity - Startle reflex - Grip strength - Nociception - Electroencephalography - Anxiety test - Visual function

Table 1: Tiered approach to safety Pharmacology evaluation (Redfern et al., 2002)

-
-
-

Bibliography

Bembo S.A., and Carlson H.E. (2004). Gynecomastia: its features, and when and how to treat it. *Cleveland Clinic Journal of Medicine* **71**, 511-517. [A short clinical review of the condition, causes and treatment]

Braunstein G.D. (1993). Gynecomastia. *N Engl J Med*. **328**, 490-495. [About drugs associated with the condition and determinants of therapy]

Briggs J.P., Kriz W. and Schnermann J.B. (2005). Overview of kidney function and structure. *Primer on Kidney Diseases* (ed. A Greenberg), 2-19. Elsevier Saunders Publications. [Describes the functional units of kidney and physiological aspects]

Buehler E.V. (1965). Delayed contact hypersensitivity in the guinea pig. *Arch Dermatol.* **91**, 171-177. [A topical application test done under occlusion]

Colpaert F.C. (1999). Drug discrimination in neurobiology. *Pharmacol Biochem Behav*. **64**, 337-345. [Discusses discriminative mechanisms and paradigms in testing for behavioral pharmacology]

Dixit R. (2004). What non-clinical toxicology and safety pharmacology data are needed to accelerate Phase I-II clinical trials. *American Pharmaceutical Outsourcing*. [The integrated plans necessary to transition a new compound entity from nonclinical to clinical development]

EMEA Guideline on the non-clinical investigation of the dependence potential of medicinal products. EMEA/CHMP/SWP/94227/2004. (issued for adoption 23 March 2006). [The European regulatory guideline for nonclinical testing of drug dependency potential]

Evans M., and Rees A. (2002). Effects of HMG-CoA reductase inhibitors on skeletal muscle: are all statins the same? *Drug Safety*, **25**, 649-663. [The range of statin-induced adverse reactions in skeletal muscles from mild myopathy to myositis, rhabdomyolysis and death are presented]

FDA (1978). Code of Federal Regulations, 21 CFR Part 58, Good Laboratory Practice for nonclinical laboratory studies. (amended/revised 1987, 1989, 1999, 2002, 2006). [The United States Food and Drugs Administration's Regulations on Good Laboratory Practices to support nonclinical studies]

Fukami M., Maeda N., Fukushige J., Kogure Y., Shimada Y., Ogawa T., and Tsujita Y. (1993). Effects of HMG-CoA reductase inhibitors on skeletal muscles of rabbits. *Res Exp Med (Berl)*. **193**, 263-273. [Studies in induction of myopathy attributable to differences in physicochemical properties of two statins]

Gad S.C. (1994). The mouse ear swelling test (MEST) in the 1990s'. *Toxicology* **93**, 33-46. [Describes the study design and methodology for the mouse ear swelling test]

Gad S.C. (2004). *Safety pharmacology in pharmaceutical development and approval*. CRC Press. [A comprehensive list of chapters and subjects covering aspects of safety pharmacology]

Greaves P. (2000). Chapter XII Endocrine System: Thyroid gland - hyperplasia induced by therapeutic agents'. *Histopathology of Preclinical Toxicity Studies - Interpretation and relevance in drug safety evaluation*, 2nd Edition, 792-795. Elsevier. [Describes hormonal and enzymatic changes in thyroid gland following administration of compounds to animals and draws attention to the need for careful designing of clinical studies to permit proper evaluation in humans]

Harris D., Graham M., Price J., Munro F., Templeton A., Young R., Paterson K., Anderson L., Gillies S., and McKendrick S. (2005). Respiratory function in rats restrained for extended periods: Assessment of

the effects of bethanecol. *J Pharmacol Toxicol Methods* **52**, 83-89. [Investigates the use of head-out plethysmography chambers for extended hours of continuous recording]

Harris J.R., and Markl J. (1999). Keyhole limpet haemocyanin (KLH): a biomedical review. *Micron*. **30**, 597-623. [Discusses the use of keyhole limpet hemocyanin in experimental and clinical immunology]

Harrison A.P., Erlwanger K.H., Elbrond V.S., Andersen N.K., and Unmack M.A. (2004). Gastrointestinal tract models and techniques for use in safety pharmacology. *J Pharmacol Toxicol Methods* **49**, 187-199. [An appraisal of the state of the art with regard to gastrointestinal assays, techniques and models]

Hart S.G.E. (2005). Assessment of renal injury in vivo. *J Pharmacol Toxicol Methods* **52**, 30-45. [An appraisal of the sate of the art with regard to renal function, and analytical and other methods to assess renal injury]

Hoffmann P., and Warner B. (2006). Are hERG channel inhibition and QT interval prolongation all there is in drug-induced torsodogenesis? A review of emerging trends. *J Pharmacol Toxicol Methods* **53**, 87-105. [Summarizes emerging trends in better understanding mechanisms of drug-induced torsadogenesis and reviews evolving preclinical methods that may assist in testing for such potential]

ICH (1997). ICH S6: Preclinical safety evaluation of biotechnology-derived pharmaceuticals. *ICH Tripartite Harmonised Guideline*. [A guideline prepared under the auspices of the International Conference on Harmonization]

ICH (2000). ICH S7A: Safety pharmacology studies for human pharmaceuticals. *ICH Harmonised Tripartite Guideline.* [A guideline prepared under the auspices of the International Conference on Harmonization]

ICH (2005). ICH E14: The clinical Evaluation of QT/QTc prolongation and and proarrhythmic potential for non-antiarrhythmic drugs. *ICH Harmonised Tripartite Guideline.* [A guideline prepared under the auspices of the International Conference on Harmonization]

ICH (2005). ICH S7B: Non-clinical evaluation of the potential for delayed ventricular repolarization (QT interval prolongation) by human pharmaceuticals. *ICH Harmonised Tripartite Guideline*, Revised Step 2 Document. [A guideline prepared under the auspices of the International Conference on Harmonization]

Irwin S. (1968). Comprehensive observational assessment: Ia A systematic, quantitative procedure for assessing the behavioral and physiologic state of the mouse. *Psychopharmacologia (Berl)* **13**, 222-257. [This paper forms the basis of the Irwin Protocol for a systematic observational assessment of behavior and aspects of compound-induced time-dose-response effects]

Jerne N.K., and Nordin A.A. (1963). Plaque formation in agar by single antibody-producing cells. *Science* **140**, 405. [Description of the experimental method]

Joshi A., Dimino T., Vohra Y., Cui C., and Yan G.X. (2004). Preclinical strategies to assess QT liability and torsadogenic potential of new drugs: The role of experimental models. *J. Electrocardiol.***37**, 7-14. [Among the models described is the rabbit left ventricle wedge preparation which is stated to correlate well with clinical outcomes]

Judy W.V., and Farrell S.K. (1979). Arterial baroreceptor reflex control of sympathetic nerve activity in the spontaneously hyperactive rat. *Hypertension* **1**, 605-611. [An investigation into the combined and individual carotid sinus and aortic baroreceptor control in the spontaneously hypertensive rat and the initiating role of the sympathetic nervous system]

Kirsch G.E., Trepakova E.S., Brimecombe J.C., Sidach S.S., Erickson H.D., Kochan M.C., Shyjka L.M., Lacerda A.E., and Brown A.M. (2004). Variability in the measurement of hERG potassium channel inhibition: effects of temperature and stimulus pattern. *J Pharmacol Toxicol Methods*, **50**, 93-101. [Testing of 15 drugs that spanned a broad range of hERG inhibition potency and pharmacological class showed that patch clamp recordings at near physiological temperatures are highly repeatable]

Lawrence C.L., Bridgland-Taylor M.H., Pollard C.E., Hammond T.G., and Valentin J.P. (2006). A rabbit Langendorff heart proarrhythmia model: predictive value for clinical identification of torsades de pointes. *Br. J. Pharmacol.* **149**, 845-860. [A validation study of the usefulness of this proarrhythmia model; provides experimental protocol and measurements]

Lu H.R., Marien R., Saels A., and de Clerk F. (2001). 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. [A comparative study of Purkinje fibers from various animal species to determine the most sensitive model]

Magnusson B. (1980). Identification of contact sensitizers by animal assay. *Contact Dermatitis* **6**, 46- 50. [Describes the guinea pig maximization test]

Magnusson B., and Kligman A.M. (1969). The identification of contact allergens by animal assay. The guinea pig Maximisation Test. *J Invest Dermatol*. **52**, 268-276. [Discusses the original concepts in experimental procedures for identification of contact allergens]

Nakahara K., Kuriyama M., Sonoda Y., Yoshidome H., Nakagawa H., Fujiyama J., Higuchi I., and Osame N. (1998). Myopathy induced by HMG-CoA reductase inhibitors in rabbits: a pathological, electrophysiological and biochemical study. *Toxicol Appl Pharmacol.* **152**, 99- 106. [Investigations by using electrophysiological, pathological and biochemical methods to study experimental myopathy]

Odum J., Lefevre P.A., Tittensor S., Paton D., Routledge E.J., Beresford N.A., Sumpter J.P., and Ashby J. (1997). The rodent uterotrophic assay: critical protocol features, studies with nonyl phenols, and comparison with a yeast estrogenic assay. *Regul Toxicol Pharmacol*. **25**, 176-188. [Describes methods that can be used to provide a sensitive and toxicologically relevant in vivo estrogenicity assessment]

OECD (1992). Guideline for Testing of Chemicals, Section 4: Health Effects, Short Term Toxicology, No: 406 Skin sensitization. [This is a guideline from the OECD]

OECD (1998)a. OECD Guidelines on Principles of Good Laboratory Practice. ENV/MC/CHEM(98)17. (revised 1997 and issued 1998). [This is a guideline from the OECD]

OECD (1998)b. Report of the first meeting of the OECD endocrine disrupter testing and assessment (EDTA) working group. ENV/MC/CHEM/RA(98)5. Paris. [This is a guideline from the OECD]

OECD (2002). OECD Guideline Series on Principles of Good Laboratory Practice and Compliance monitoring. (Internet reference - http://www.oecd.org/document/63/0,2340,en_2649_34381_2346175_1_1_1_1,00.html). [This is a guideline from the OECD]

OECD (2003). Draft Report of the validation of the uterotrophic Bioassay: Phase 2. Testing of potent and weak oestrogen agonists by multiple laboratories. *Task Force on Endocrine Disrupters Testing and Assessment (EDTA) of the Test Guidelines Programme*. ENV/JM/TG/EDTA. [This is a guideline from the OECD]

OECD (2004). Series on Principles of Good laboratory Practice and Compliance Monitoring, No 14: Advisory Document of the working Group on Good Laboratory Practice; The Application of the Principles of GLP to in vitro Studies. ENV/MJ/MONO. [This is a guideline from the OECD]

OECD (2006). Series on Testing and Assessment, No. 59: The validation of the enhanced test guideline 407 repeat dose 28-day oral toxicity study in laboratory rats. (Internet reference - http//www.oecd.org/dataoecd/56/24/37376909.pdf). [This is a guideline from the OECD]

Official Journal of the European Communities (1992). B.6. Skin Sensitization. *L383A* **35**, 131-139. [Describes experimental methods for the testing of skin sensitization potential and a classification based on results]

Overton D.A. (1987). Applications and limitations of the Drug Discrimination Method for the study of drug abuse. *Methods of assessing the reinforcing properties of abused drugs* (ed. MA Bozarth), 291-340. New York: Springer-Verlag. [Describes the advantages and disadvantages of drug discrimination procedures as methods for obtaining information about the properties of drugs that underlie drug abuse]

Owens W., Gray Jr L.E., Zeiger E., Walker M., Yamasaki K., Ashby J., and Jacob E. (2007). The OECD program to validate the Rat Hershberger bioassay to screen compounds for in vivo androgen and antiandrogen responses: Phase 2 dose-response studies. *Environ Health Perspect.* **115**, 671-678. [Provides results and conclusions from an inter-laboratory validation study on the reproducibility and robustness of the OECD Hershberger assay]

Pugsley M.K. (2005). Methodology used in safety pharmacology: Appraisal of the state-of-the-art, the regulatory issues and new directions. *J Pharmaacol Toxicol Methods,* **52**, 1-5. [An editorial for a

dedicated issue of the journal devoted exclusively to methodology, opinions and regulatory issues related to safety pharmacology]

Redfern W.S, Carlsson S., Davis A.S., Lynch W.G., MacKenzie I., Palethorpe S., Siegl P.K., Strang I., Sullivan A.T., Wallis R., Camm A.J., and Hammond T.G. (2003). Relationships between preclinical cardiac electrophysiology, clinical QT interval prolongation and torsades de pointes for a broad range of drugs: evidence for a provisional safety margin in drug development. *Cardiovasc Res.* **58**, 32-45. [This paper is a review of published literature on nonclinical and clinical data on an initial list of 100 drugs exploring the relationships]

Redfern W.S., Strang I., Storey S., Heys C., Barnard C., Lawton K., Hammond T.G., and Valentin J.P. (2005). Spectrum of effects detected in the rat fubnctional observational battery following oral administration of non-CNS targeted compounds. *J. Pharmacol Toxicol Methods* **52,** 77-82. [Provides a systematic assessment of 50 compounds not targeting CNS disorders, in the rat functional observational battery to assess the frequency of requirement for different follow up studies]

Redfern W.S., Wakefield I.D., Prior H., Pollard C.E., Hammond T.G., and Valentin J.P. (2002). Safety pharmacology – a progressive approach. *Fundamental & Clinical Pharmacology* **16**, 161-173. [An informative overview of the aims, safety pharmacology data collation, and use in risk assessment]

Sarlo K., Fletcher E.R., Gaines W.G., and Ritz H.L. (1997). Respiratory allergenicity of detergent enzymes in the guinea pig intra-tracheal test: association with sensitization of occupationally exposed individuals. *Fundamental and Applied Toxicology* **39**, 44-52. [Describes an animal model for evaluating enzymes as respiratory allergens]

Satoh T., Fujita K.I., Munakata H., Itoh S., Nakamura K., Kamataki T., Itoh S., and Yoshizawa I. (2000). Studies on the interactions between drugs and estrogen: analytical method for prediction system of gynecomastia induced by drugs on the inhibitory metabolism of estradiol using Escherichia coli coexpressing human CYP3A4 with human NADPH-cytochrome P450 reductase. *Anal. Biochem.* **286**, 179-186. [A system based on the assumption that 50% inhibition concentration of drugs on the *in vitro* metabolism of estradiol to its major product 2-hydroxyestradiol can be substituted as a prediction index for gynecomastia]

Satoh T., Tomikawa Y., Takanashi K., Itoh S., Itoh S., and Yoshizawa I. (2004). Studies on the interactions between drugs and estrogen. III. Inhibitory effects of 29 drugs reported to induce gynecomastia on the glucuronidation of estradiol. *Biol. Pharm.Bull.* **27**, 1844-1849. [Emphasizes the contribution of Phase II reaction (conjugation) as one of the reactions affecting the estrogen pool]

Schlede E., and Eppler R. (1995). Testing for skin sensitization according to the notification procedure for new chemicals: the Magnusson and Kligman test. *Contact Dermatitis* **32**, 1-4. [This paper gives recommendations for standardized performance of the Magnusson and Kligman test]

Shay H., Komarov S.A., Fels S.S., Meranze D., Gruenstein M., and Siplet H. (1945). A simple method for the uniform production of gastric ulceration in the rat. *Gastroenterology* **5**, 43-61. [A modified technique useful in studying gastric secretion and inflammation]

Smith P.F., Eydelloth R.S., Grossman S.J., Stubbs R.J., Schwartz M.S., Germershausen J.I., Vyas K.P., Kari P.H., and MacDonald J.S. (1991a). HMG-CoA reductase inhibitor-induced myopathy in the rat: cyclosporine A interaction and mechanistic studies. *J Pharmacol Exp Ther*. **257**, 1225-1235. [Provides experimental evidence for potentiation of myopathy by HMGCoA reductase inhibitors when co-administered with drugs that alter systemic clearance and increase tissue exposure]

Smith P.F., Grossman S.J., Gerson R.J., Gordon L.R., Deluca J.G., Majka J.A., Wang R.W., Germershausen J.I., and MacDonald J.S. (1991b). Studies on the mechanism of simvastatin- induced thyroid hypertrophy and follicular cell adenoma in the rat. *Toxicol Pathol.* **19**, 197- 205. [Explores if a relationship exists between hepatic metabolism and thyroid cell changes under experimental conditions]

Touvay C., and Le Mosquet B. (2000). The respiratory system and safety pharmacology [In French]. *Therapie* **55**, 71-83. [Describes the safety pharmacology methods used for routinely investigating respiratory function and also discusses the usefulness of studying function under pathological conditions]

Uetrecht J. (2006). Role of animal models in the study of drug-induced hypersensitivity reactions. *AAPS Journal* **7**, E914-E921. [Describes animal models for mechanistic studies and screening assays]

Vogel H.G., Hock F.J., Maas J., and Mayer D. (2006). Section 1- Safety Pharmacology. *Drug Discovery and Evaluation: Safety and Pharmacokinetic Assays.* Springer. [A book covering subjects on safety pharmacology, safety pharmacokinetics and safety toxicology]

Biographical Sketches

Dr Jega Iswaran received a PhD degree from the University of London, and the Membership (MRCPath) and Fellowship (FRCPath) Diplomas of the Royal College of Pathologists (UK), specializing in toxicology and pathology. In the pharmaceutical industry, he has worked in various research and product development positions over a 30 year period. During his long tenure at ICI/Zeneca Pharmaceuticals, at the company's headquarters in England, he held senior positions of authority in the fields of safety pharmacology, toxicology and pathology and was actively associated with several international product development programs covering the therapeutic areas of cancer, endocrinology, infection, cardiovascular disease, CNS disease, gastrointestinal disease and respiratory disease. In this capacity, he also acted as company expert on matters of drug safety evaluation. Since 1996, he has lived in Australia and worked in the pharmaceuticals sector in Melbourne, first at CSL Bioplasma Limited and then as Development Director at Antisense Therapeutics limited. His current interests are in the areas of drug safety of antiviral agents.

Professor Jorma Ahokas is a pharmacologist/toxicologist by training. He received a PhD in Finland and did a postdoc as a Merck Sharp & Dohme Research Fellow in Clinical Pharmacology in the Department of Medicine, University of Queensland, followed by an Australian National Health & Research Council funded research post. After lectureships in the Department Pharmacology, University of Melbourne and RMIT, in 1991 he was appointed to a position of Foundation Professor in Toxicology at RMIT. It was the first full professorship in toxicology in Australia. Since 1998, he has been a Docent in toxicology at the University of Helsinki and since 1999 a visiting professor of toxicology at Toho University, Japan. His research has related to problems of drug and carcinogen metabolism as well as food and environmental toxicology. His current research interests relate to adverse effects of complementary remedies and their interactions with prescription medicines. He has been a consultant in areas of drug toxicity and environmental toxicology to industry and government bodies. Jorma Ahokas is a Professor of Toxicology, School of Medical Sciences, RMIT University.