Institution: Imperial College London

Unit of Assessment: 01 Clinical Medicine

Title of case study: Development of a Rational Framework to Evaluate the Toxicity of Drugs and Chemicals in Food and the Environment.

1. Summary of the impact (indicative maximum 100 words)

The safety assessment of drugs and other chemicals relies upon studies in experimental animals. Whilst these are useful surrogates, extrapolation to humans requires several assumptions. Professor Boobis led an international group under the auspices of the World Health Organisation (WHO), to develop a framework for the systematic and transparent assessment of such experimental data. Within this framework, the toxicological effect of a chemical is broken down into a series of intermediate steps, comprising a mode of action. This enables qualitative and quantitative comparison between experimental animals and humans. The framework has impacted on risk assessment policy both nationally and internationally, on product development, and on risk assessments of combined exposure to chemicals.

2. Underpinning research (indicative maximum 500 words)

Key Imperial College London researchers:
Professor Alan Boobis, Professor of Biochemical Pharmacology (1979-present)
Professor Donald Davies, Professor of Biochemical Pharmacology (1967-2005)
Dr Robert Edwards, Research Lecturer (1984-present)
Professor Nigel Gooderham, Professor of Molecular Toxicology (1985-present)
Dr Hazel Jones, Senior Postdoctoral Fellow (1970-2009)
Dr Stephen Murray, Senior Research Officer (1973-2006)

A key event in the toxicity of many chemicals is their metabolic activation by P450 enzymes. Work by Professor Boobis and Imperial colleagues, in the late 1990s (1) on these enzymes led to the development of a unique strategy for the quantification and localisation of specific forms of P450 in cells and tissues. This strategy was based on the use of short (~4-5 amino acid residues), often C-terminal, peptides as haptens for generation of P450-form specific antibodies. Such antibodies were invaluable in quantification of P450 enzymes involved in the metabolic activation of chemicals, such as heterocyclic amines in cooked meat, polycyclic aromatic hydrocarbons, and chlorination by-products such as chloroform. Application of the antibodies enabled tissue, species, life-stage and inter-individual differences in P450 expression to be determined and applied in risk assessment.

Information derived from studies on P450 enzymes involved in the metabolism of heterocyclic amines in cooked meat (2) was combined with results from a variety of genotoxicity studies and estimates of human exposure, obtained over a number of years in our laboratory, to assess likely human risk from the presence of these amines in the diet.

Work in the mid-2000's led to the publication by Professor Boobis of a human relevance-mode of action framework for assessing chemical carcinogens (3). The human relevance mode of action framework was later extended to cover non-cancer endpoints (4). This framework, published in 2008, concluded that essentially all toxicological responses could be described as a series of essential, quantifiable changes in biochemical or physiological processes. Individually each change is necessary, but not sufficient to elicit a toxic response, but, collectively, they comprise a mode of action. Each event is quantifiable and it is thus distinct from mechanism of action, which implies a more detailed molecular understanding of the effect, where many of the hypothesised steps may not be quantifiable. By comparing key events in experimental animals and humans, in vitro or in vivo, qualitative and quantitative assessments of the key events can be undertaken. This enables the human relevance of observations in experimental animals to be assessed and provides a scientifically-defensible basis for data-derived extrapolation.

In an international collaboration coordinated by the Research Foundation of the International Life Sciences Institute, in which Professor Boobis played a lead role in the chemicals core group (5), the quantitative assessment of key events in a mode of action to analyse dose response

relationships was shown to apply not only to chemical toxicity and carcinogenicity, but also to nutrients, allergens and microbial pathogens. This provided unique insight into the risk assessment of a diverse range of food-borne stressors, helping in the quantitative extrapolation of risks from experimental studies to humans and in the identification of susceptible sub-populations.

The application of the human relevance-mode of action concept has been explored in the risk assessment of combined exposure to multiple chemicals in a series of international collaborations in the late 2000's, in which Professor Boobis played a key role (6). This enabled the development of a tiered approach to such risk assessment, based on increasing refinement of both exposure estimates and knowledge of mode of action, thus allowing more efficient use of time and resources, whilst also enabling ready identification of key knowledge gaps.

3. References to the research (indicative maximum of six references)

(1) Edwards, R.J., Adams, D.A., Watts, P.S., Davies, D.S., & Boobis, A.R. (1998). Development of a comprehensive panel of antibodies against the major xenobiotic metabolising forms of cytochrome P450 in human. *Biochem. Pharmac*., 56, 377-387. DOI. Times cited: 103 (as at 23rd October 2013 on ISI Web of Science). Journal Impact Factor: 4.57

(2) Boobis, A.R., Lynch, A.M., Murray, S., de la Torre, R., Solans, A., Farre, M., Segura, J., Gooderham, N.J., & Davies, D.S. (1994). CYP1A2 catalyzed conversion of dietary heterocyclic amines to their proximate carcinogens is their major route of metabolism in humans. *Cancer Res*., 54, 89-94. Times cited: 205 (as at 23rd October 2013 on ISI Web of Science). Journal Impact Factor: 8.65 (available from http://cancerres.aacrjournals.org/content/54/1/89.full.pdf)

(3) Boobis, A.R., Cohen, S.M., Dellarco, V., McGregor, D., Meek, M.E., Vickers, C., Willcocks, D., & Farland, W. (2006). IPCS framework for analysing the relevance of a cancer mode of action for humans. *Crit. Rev. Toxicol*., 36, 781-792. DOI. Times cited: 117 (as at 23rd October 2013 on ISI Web of Science). Journal Impact Factor: 6.25

(4) Boobis, A.R., Doe, J.E., Heinrich-Hirsch, B., Meek, M.E., Munn, S., Ruchirawat, M., Schlatter, J., Seed, J., & Vickers, C. (2008). IPCS framework for analyzing the relevance of a noncancer mode of action for humans. *Crit Rev Toxicol,* 38, 87-96. DOI. Times cited: 73 (as at 23rd October 2013 on ISI Web of Science). Journal Impact Factor: 6.25

(5) Boobis, A.R., Daston, G.P., Preston, R.J., & Olin, S.S. (2009). Application of key events analysis to chemical carcinogens and noncarcinogens. *Crit Rev Food Sci Nutr,* 49, 690-707. DOI. Times cited: 23 (as at 23rd October 2013 on ISI Web of Science). Journal Impact Factor: 4.82

(6) Meek, M.E., Boobis, A.R., Crofton, K.M., Heinemeyer, G., Raaij, M.V., & Vickers, C. (2011). Risk assessment of combined exposure to multiple chemicals: A WHO/IPCS framework. *Regul Toxicol Pharmacol,* 60, S1-S14. DOI. Times cited: 16 (as at 23rd October 2013 on ISI Web of Science). Journal Impact Factor: 2.13

Key Funding:

- Ministry of Agriculture, Fisheries and Food (MAFF) (1994-1997; £102,000 p.a.), Principal Investigator (PI) A. Boobis and N. Gooderham, Assessment of human exposure to reactive metabolites of dietary genotoxins.
- MAFF (1997-2000; £104,000 p.a.), PI A. Boobis and N. Gooderham, Can biomarkers be used to assess the carcinogenic potential of heterocyclic amines?
- Department of Health/Health Protection Agency (1998-2014; £9,850,000), PIs D. Davies, A. Boobis, Support of Toxicology Unit.
- Biotechnology and Biological Sciences Research Council (BBSRC) (2002-2005; £373,236), PIs R. Edwards and A. Boobis, A universally applicable approach for the generation of protein-specific antibodies: applications in proteomics.
- Medical Research Council (MRC) (2009-2012; £529,215), PIs A. Boobis, S. Grimm, R. Edwards, and T. Tetley), Pathway analysis in characterising the toxicological properties of nanomaterials.

4. Details of the impact (indicative maximum 750 words)

Impacts include: health and welfare, public policy and services, production, international development
Main beneficiaries include: Government, consumers and manufacturers, risk assessment bodies, including the US Environmental Protection Agency (EPA), UK Scientific Advisory Committees, the National Health and Medical Research Council of Australia, the European Food Safety Authority (EFSA), the Commission of the European Communities, and the WHO

The human relevance-mode of action framework, developed by Professor Boobis and colleagues, provides a systematic and transparent approach to assessing the relevance to humans of findings in experimental animals on the toxicity of chemicals in food and the environment, which has been widely adopted by risk assessment bodies. It supports the quantitative extrapolation of such findings to humans, using specific data, as opposed to defaults, where appropriate. Previously, such extrapolations lacked transparency and international consensus.

Policy and risk assessment: The Joint WHO/Food and Agriculture Organisations (FAO) Expert Committees on Food Additives (JECFA) and Pesticide Residues (JMPR) undertake risk assessments of a number of types of chemicals that may be found in food. These include food additives, residues of veterinary drugs, residues of pesticides, and chemical contaminants of natural or synthetic origin. The outcome of these risk assessments are the basis for harmonised, safety-based, worldwide trading standards at the Codex Alimentarius. This allows the international trade of food commodities, whilst ensuring consumer safety. The value of the framework is illustrated by the risk assessment of the pesticide sulfoxaflor by JMPR in 2011 and of the artificial sweetener aspartame by European Food Safety Authority (EFSA) in 2011 [1]. The application of the framework enabled the conclusion that several of the toxicological effects observed in experimental animals were not relevant to humans and thus it was possible to identify exposure levels in food consistent with the safe use of the compounds [1].

The principles and methods for the risk assessment of chemicals in food, used by the WHO/FAO expert committees, member states and others undertaking such work, including producers and manufacturers, are published in Environmental Health Criteria (EHC). These criteria were revised in 2009 to acknowledge the importance of considering mode of action using the underpinning Imperial framework [2]. Others who have used the framework in risk assessment policy include the US EPA, the UK Scientific Advisory Committees, the National Health and Medical Research Council of Australia, the European Food Safety Authority and the Commission of the European Communities [3].

Animal welfare: Use of the framework is contributing to the reduction, refinement and replacement of animals in toxicity testing. Prior to human exposure, chemicals are assessed for their potential toxicity. The extent of testing depends on the intended use of the chemical and the relevant legislation. In order to avoid unnecessary testing, the Organisation for Economic Co-Operation and Development (OECD) has produced a series of test guidelines, which are internationally accepted. In general, results of a study conducted according to such a guideline can be used to support authorisation or approval in any member country. In the 2009 revision of the test guideline for studying the potential long term toxicity and carcinogenicity of chemicals, the OECD emphasised the importance of obtaining information in such studies that would help determine mode of action the relevance of experimental findings and cite the underpinning work of Professor Boobis and colleagues as an important source of information in such design considerations [4].

In assessing the risk of combined exposure to chemicals, it is important to consider mode of action, as chemicals sharing a mode of action will exhibit dose addition, and hence it is important to consider such compounds in a common group. Several recent activities have highlighted the value of the mode of action framework for establishing such common assessment groups. Examples include EFSA's Scientific Panel on Plant Protection products and their Residues in 2008 [5], the WHO International Programme on Chemical Safety (IPCS) in 2009 [6], and the non-food committees of the General Health and Consumers Directorates (DG SANCO) [7].

Manufacturers: The risk assessment of a number of substances has been improved by the generation of data on mode of action and key events by companies, using the above framework as a guide to determine data needs. For example, the company producing the pesticide fluopicolide used the framework to generate specific information on mode of action, which was essential in its evaluation by the FAO/WHO JMPR in 2009 [8].

The antibodies and the approach to their generation using short specific peptides, in which specificity can be confirmed by sequence scanning of relevant genomes, have seen widespread application in many areas, including several relevant to the use of human relevance-mode of action analysis. In addition to work undertaken in our own laboratory and in collaboration with others, the antibodies are commercially available through companies such as Daiichi (overall sales of >£150,000) and our strategy has been used by companies such as BD Gentest to raise similar antibodies. An example of the use of the output of our studies using such antibodies can be found in the US EPA report "Exploration of Perinatal Pharmacokinetic Issues" [9]. The antibodies have been of particular value for localisation and quantification of P450 enzymes. More widely, the approach has allowed the generation of specific antibodies to peptides or proteins, where other approaches did not permit the necessary specificity.

Training: The framework has been presented at a number of workshops, to increase awareness and expertise amongst the risk assessment community. This has been invaluable in disseminating the value and application of the framework in a wide variety of situations [10].

5. Sources to corroborate the impact (indicative maximum of 10 references)

[1] Risk assessment of Sulfoxaflor pesticide by JMPR in 2011 (see pages 653 & 754; archived on 23rd October 2013) and aspartame by EFSA (see pages 10-12; archived on 23rd October 2013).

[2] EHC 240, World Health Organisation, Geneva, 2009 (ISBN 978 92 4 157240 8; available online (see pages 4-14, 4,17, 4-76). Archived on 23rd October 2013.

[3] Examples of the use of the framework for risk assessment policy:
(i) US EPA white paper on Predicting the Toxicities of Chemicals to Aquatic Animal Species (2010) see pages 18, 57 (archived on 23rd October 2013)
(ii) UK Scientific Advisory Committees (e.g. http://cot.food.gov.uk/pdfs/cotsection07.pdf) Archived on 23rd October 2013
(iii) European Food Safety Authority (archived on 23rd October 2013)
(iv) Commission of the European Communities (archived on 23rd October 2013)

[4] OECD Test Guideline No. 453: Combined Chronic Toxicity/Carcinogenicity Studies, adopted 7/9/09 (DOI; see page 2)

[5] The EFSA Journal (2008) 704, 12-84 (see page 79, archived on 23rd October 2013)

[6] WHO/IPCS framework for assessing risk from combined exposures to multiple chemicals (2009; see pages 14, 31, and 38). Archived on 23rd October 2013.

[7] Opinion of DG SANCO scientific committees on Toxicity and Assessment of Chemical Mixtures (2011; see pages 17, 20, and 33). Archived on 23rd October 2013.

[8] FAO/WHO JMPR evaluation of fluopicolide (2009; see page 348). Archived on 23rd October 2013.

[9] US EPA report "Exploration of Perinatal Pharmacokinetic Issues". Archived on 23/10/2013.

[10] Training examples include: US Society of Toxicology Continuing Education Course (2009; archived on 23/10/13), WHO/OECD Training Workshop, Paris (see page 3; archived on 23/10/13), World Congress on Risk, Sydney (2012; archived on 23/10/13).