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Dosing Data: Volume & Frequency

Dosing Limit Volumes: The United Kingdom View - Past and Present

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Summary

The Home Office believes that by standardising procedures, and identifying, eliminating or minimising unwanted physiological and pathological effects, good science and good welfare are safeguarded.

This paper reviews the development and application of recommended limited dosing volumes introduced by the Home Office, and explores other refinement related aspects of dosing protocols.

Introduction

Throughout the European Union, the use of animals for experimental and other scientific purpose is regulated by the European Union Council Directive 86/609 EEC (Official Journal of the European Communities, 1986). In the United Kingdom this has been transposed into domestic legislation as the Animals (Scientific Procedures) Act 1986 (HMSO, 1990).

Under the terms of the Directive, and the 1986 Act, the use of living animals is permissible only when replacement alternative methods are not reasonably or practically available, and once every reasonable effort has been demonstrably made to maximise the quality of the science and minimise the pain and distress experienced by the animals used. The concepts of the 'Three Rs' of replacement, reduction and refinement (Russell & Burch, 1959) are central to the operation of the regulatory framework.

The 1986 Act applies regulation at three levels: the research establishments conducting animal experiments, the individual programmes of work, and the personnel who interact with the animals. At any one time the Home Office regulates in excess of five thousand programmes at work, carried out at over three hundred research establishments, by over fifteen thousand researchers. In addition a Home Office Inspectorate, staffed by medical and veterinary graduates with personal experience of research in the life sciences, referees all applications for authorities under the Act and performs in excess of two thousand visits of inspection per year to establishments where the research is

conducted. The Home Office thus has access to comprehensive and detailed information on the animal based research activity in the United Kingdom.

The Home Office subjects all proposals for the use of living animals for experimental or other scientific purposes to a cost/benefit assessment. Only those applications where the objectives are likely to be realised, and where the potential, realistic benefits exceed the likely welfare cost proceed further through the regulatory systems. Details of the cost/benefit assessment have been published (The Stationary Office, 1998).

The Home Office also plays active educational and representational roles in identifying and disseminating best practice with respect to science and animal welfare. A range of best practice 'minimum severity' protocols have been produced covering subjects including antibody production, the use of animals for education and training, eye safety tests, the use of neuromuscular blocking agents and the production of genetically modified animals. Furthermore, the Home Office supplies technical experts to service third party reduction, refinement and replacement initiatives, and actively funds research in these areas.

Background

The administration of test materials is an integral part of most animal-based research protocols. It is a surprisingly complex area both from the perspective of ensuring the scientific validity of the research, and safeguarding the welfare of the animals used. Refinement and standardisation of protocols for the administration of substances are essential to safeguard science and welfare.

Table 1 illustrates the complexity of this area by listing only some of the factors relating to the administration of test materials that influence the quality of the science, the physiological responses and the welfare costs. These must be considered in the context of the diverse circumstances where animals are dosed with biologically active substances, often when the likely nature of the activity is relatively unknown, by many routes and for numerous purposes.

Table 1		
Factors Related To Administration of Test Substances		
The Experimental Subject	The Test Material	Other
Species	Biological Activity	Purpose
Strain	Biocompatibility	Route
Stage of development	Formulation	Technical competence
Weight	pH/pK/Buffer capacity	Technology
Age	Bioavailability	Timing
Gender	Stability	Duration
State of health	Sterility	Frequency
Diet	Chemical form	Rate of delivery
Husbandry	Tonicity	Site
Fasting	Toxicokinetics	Previous exposure
Anaesthesia	Toxicodynamics	Staff safety
Restraint	Temperature	Portal/pulmonary clearance
Timing and habituation	Viscosity	Vehicle
And Dosing Volume		

Science and Welfare

The administration of test substances to animals is a complex area: the parameters set must ensure good science and must not unintentionally compromise the welfare of the experimental subjects.

Good science requires the consistent application of test methods well matched to specific research objectives: this includes the identification and elimination or acknowledgement of unwanted variables.

The principles of humane research require that all reasonable efforts be made to minimise or eliminate unwanted physiological or pathological effects. Care is needed to ensure that protocol refinement does not compromise or invalidate the science. It is important that consideration is given at an early stage of compound development or assessment to the production and use of formulations that bear these principles in mind.

Limit Volumes

Ten years ago, a Home Office review of technical authorities sought under the 1986 Act highlighted dramatic differences in the proposed limit volumes for dosing (and blood sampling). This raised concerns about the rational basis for such practices and the possibility that science or welfare might, as a result, be compromised.

Good science requires that variables, other than those that are the object of the study, be identified and ideally eliminated - or at least acknowledged and controlled. These include unwanted physiological and pathological effects resulting from regimens used for the administration of test materials. Good science requires a clear understanding of the consequences of the interventions, and the highest welfare standards.

Volume Related Effects

The volumes administered may, of themselves, produce unwanted and often unrecognised, physiological or pathological changes which can influence the nature and interpretation of the research findings. Pain and discomfort (and the associated autonomic, neuro-endocrinological and behavioural changes) other volume related physiological effects, such as haemodilution and cardiovascular or renal changes, can alter the animals' apparent response to the test material and invalidate any conclusions drawn.

Furthermore the volumes administered, and the rate of administration, may influence the bioavailability of the test material (Svendson & Kornerup Hansen 1998), and with larger volumes effects may be related to the biological or other properties of the vehicle used rather than the test material. Even 'physiological saline' is not a physiological fluid. The importance of the correct, informed choice of vehicle is covered elsewhere in this meeting.

Route of Administration

The route of administration has profound effects on the bioavailability of the test material (Svendson & Kornerup Hansen, 1998).

The route of administration may also place physical limits on the volumes that can be administered. The intradermal and intramuscular routes provide no natural space: intra-articular and intracerebral administration access limited spaces; intravascular administration may allow rapid access to other fluid compartments: gavage dosing gives access to the upper gastrointestinal tract; subcutaneous administration accesses a variable sized potential space; and intraperitoneal administration accesses a larger potential space although it does not mimic any common natural route of exposure and allows the test material to partition in an uncontrolled manner between the portal and systemic circulations.

With dietary administration, or intranasal dosing, limit volumes are seldom a major issue, rather limitations are determined by accuracy of dosing, dose exposure and rate and time of exposure.

Routes of administration should be chosen to minimise welfare problems, but should not be adjusted to conceal welfare problems: in welfare terms there seems no benefit in using the intramuscular route of administration for materials known to cause severe irritation or ulceration when administered by other routes.

Home Office Guidance On Limit Volumes For The Administration of Substances

The Home Office initially set out to develop evidence-based internal guidelines defining acceptable dosing limit-volumes consistent with good science and good welfare. The limited, primary intention was to ensure that members of the Home Office Inspectorate offered defensible, consistent advice on requests for technical authorities.

The challenge was seen to be to produce a consensus guidance document on limit volumes for administration of test materials based upon evidence of best science and best welfare or, in the absence of such data, current perceived best practice. Such a document required to be sufficiently flexible to allow for the many other relevant factors to be taken into consideration (See Table 1): thus the intention was not to set absolute maxima for all circumstances. Rather the emphasis was on justification for the volumes to be used, and to promote continuous improvement.

The exercise was also used as an opportunity to foster an understanding of the many factors requiring to be considered, and their interaction, and to reinforce that importance of good science and good welfare.

An analysis of existing technical authorities issued under the 1986 Act, and a knowledge of in-house practices at research establishments, was supplemented by a review of the relevant clinical, scientific and welfare literature which produced a plethora of often contradictory and unreferenced thought, opinion and advice, but demonstrated a lack of evidence-based limit volumes for the administration of substances to animals. This was also the outcome of a wider consultation with a range of experts. On scientific and welfare grounds, some practices described could not be defended. It was also confirmed that some of the extreme limits described were never actually used in practice.

From this it became clear that perceived 'best practice' would determine the contents of the resulting guidelines.

However, there was sufficient commonality amongst the practices of a number of major companies for a defensible, consensus set of baseline limit volumes for dosing to be established, with

provision being made for occasional exceptions where good cause could be demonstrated. It was clear that, particularly for intradermal and intramuscular administration, lower volumes might have to be offset against the use of more sites.

As a result an internal Home Office guidance document was produced, based upon perceived best United Kingdom practice, to assist inspectors determine the reasonableness, or otherwise, of requests for technical authorities: with the rider that the biological effects of the test materials and other variables, such as those listed in Table 1, also had to be considered when these judgements were made.

In due course, the Home Office 'requirements' became known to those seeking authorities under the 1986 Act.

Table 2 reproduces the original limits wet for dosing volumes.

Table 2: Home Office Guidelines: Limit Volumes for Administration of Substances

	ORAL mls/kg	SC mls/kg	IP mls/kg	IM ** mls/site	IV mls/kg	ID** mls/site
Mouse	20 (50)	50	20	0.05 (0.1)	10 (25)	0.05
Rat/Hamster	20 (30)	5	10	0.1 (0.2)	5 (20)	0.1
Guinea Pig	20	5	10	0.1 (0.2)	5 (20)	0.1
Rabbit	2 (10)	1	4	0.2 (0.5)	2 (8)	0.1
Cat	1	0.6	1	0.2 (0.5)	2.5 (3)	0.1
Dog	10	0.6	1	0.2 (0.5)	2.5 (3)	0.1
Fowl	2	0.5	0.5	0.2 (0.5)	2.5 (3)	0.1

() Volumes in parenthesis = exemptions accepted under specific circumstances

** For the intramuscular and intradermal routes, where there is limited natural space, the volumes specified are the maximum for any single site: for other routes, limits are expressed in mls/kg.

Separate, more detailed, guidance was produced for specific circumstances such as the use of adjuvants and eye safety testing.

In Practice

This general guidance set the scene for consistent application of policy and standards by the Home Office and the rapid convergence of practices towards these norms.

In general the guidance was well received - not least because industry and academia had been consulted or informed at various stages, and the limits set were already known to allow a number of key establishments to operate successfully. Thus, in the event, the fact that the material was clearly based upon best existing practice, rather than having been derived from an untried theoretical ideal, helped with acceptance. A generally 'level playing field' was established for users, and a 'safety-net' provided for inexperienced users.

The existence of the guidance has not inhibited progress towards further refinement, and does not appear to have selectively disadvantaged the UK research community.

Requests to exceed the stated maxima have not been common. Many applicants do not seek authorities at the upper limits, and indeed the upper limits are seldom used in practice. Information is collated centrally on justifiable exceptions to these general requirements, and of instances where there is documentary evidence of science or welfare being compromised even within these limits. Problems with the use of concentrated solutions used to stay within these volumes limitations but producing problems with biocompatibility or bioavailability have not been commonly observed.

The Present

The Home Office guidance is now ten years old and due for revision. The existing guidance is silent on some newer technologies, such as continuous infusion and other systems requiring surgical preparation, and needs to better integrate advice on limit volumes with insights into the significance of the other variables.

LASA (the Laboratory Animal Science Association) has recently produced 'Good Practice Guidelines' for the 'Administration of Substances' (LASA, 1998) dealing very briefly with limit volumes and other aspects of dosing. Its recommendations mirror the current Home Office guidance.

A forthcoming UK BVAAWF/FRAME/RSPCA/UFAW Joint Working Group on Refinement Report entitles 'Refining Procedures For the Administration of Substances will, in addition to reviewing limit volumes, consider the interaction of some of the factors itemised in Table 1.

The European Federation of Pharmaceutical Industries in the process of completing its own review and guidance: this will be discussed elsewhere in this meeting.

Conclusion

There is still a need for evidence-based internationally acceptable limit volume guidelines, and for those involved in animal-based research to recognise and acknowledge the scientific and welfare issues surrounding protocols for the administration of substances, and the complexity of the considerations.

The publication of guidelines should not inhibit the drive for continuous improvement in terms of reduction, refinement and replacement alternatives and better science.

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