# *Guidance for the use and reporting of anaesthetic agents in BJP manuscripts involving work with animals*

Article

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**Guidance for the use and reporting of anaesthetic agents in BJP manuscripts involving work with animals**

Jerry Ingrande[1,*], Hemal H. Patel[1,*], Dave Kendall[2], Barbara Stefanska[3], Steve Alexander[2], Mick Bakhle[4], Giuseppe Cirino[5], James R. Docherty[6], Christopher H. George[7], Paul A. Insel[8], Yong Ji[9], Brian F. King[10], Elliot Lilley[11], Reynold A. Panettieri, Jr.[12], Andrew G. Ramage[10], Christopher G. Sobey[13], S. Clare Stanford[10], Gary Stephens[14], Mauro Teixeira[15], Nathalie Vergnolle[16] and Amrita Ahluwalia[17]

Editorial Office
British Journal of Pharmacology

Correspondence:
A Ahluwalia
British Journal of Pharmacology
The Schild Plot
16 Angel Gate
City Road
London
EC1V 2PT | UK

Tel: +44 (0) 20 7239 0171
Fax: +44 (0) 20 7417 0114
E-mail: info@bps.ac.uk

**Author affiliations:**
[1] VA San Diego Healthcare System and University of California, San Diego, USA
[2] University of Nottingham, UK
[3] The University of British Columbia, Vancouver, Canada
[4] Imperial College London, UK
[5] University of Naples Federico II, Italy
[6] Royal College of Surgeons in Ireland, Dublin, Ireland
[7] Swansea University, UK
[8] University of California, San Diego, USA
[9] Nanjing Medical University, Nanjing, China
[10] University College London, UK
[11] NC3Rs, London, UK
[12] Rutgers University, New Jersey, USA
[13] La Trobe University, Australia
[14] University of Reading, UK
[15] Federal University of Minas Gerais, Belo Horizonte, Brazil
[16] INSERM, Toulouse, France
[17] Queen Mary University of London, London, UK
[*] Contributed equally to the work.

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**Abbreviations**

ACTH, adrenocorticotropic hormone
BJP, British Journal of Pharmacology
GABA, gamma aminobutyric acid
GFR, glomerular filtration rate
MAC, minimum alveolar concentration
MAP, mean arterial pressure
NMDA, N-methyl-D-aspartate
PAM, positive allosteric modulator
BBF, renal blood flow

**Contributions of authors**

This article originated from discussions at meetings of the Senior Editors of BJP with the EiC and the full Editorial Board during 2019 to 2021. Jerry Ingrande and Hemal H. Patel contributed equally to the original draft of this manuscript.

**Abstract**

Scientists who plan to publish in the *British Journal of Pharmacology* (*BJP*) must read this article before undertaking studies utilizing anaesthetics in mammalian animals. This editorial identifies certain gaps in the reporting of details on the use of anaesthetics in animal research studies published in *BJP*. The editorial also provides guidance, based upon current best practices, for performing in vivo experiments that require anaesthesia. In addition, mechanisms of action and physiological impact of specific anaesthetic agents are discussed. Our goal is to identify best practices and to provide guidance on the information required for manuscripts submitted to *BJP* manuscripts that involve the use of anaesthetic agents in studies with experimental animals.

## Introduction

The inadequate reporting of methods used for in vivo animal experimentation has been identified as a key deficiency in the literature of discovery science research, particularly within the biomedical field. This deficiency directly impacts on replicability and translatability. The development, and most importantly use, of guidelines to support researchers in understanding current best practice has been shown to improve this problem. Examples include the ARRIVE 2.0 guidelines and *BJP* guidance (Lilley et al., 2020; Percie du Sert et al., 2020). Amongst the list of essential details required to enable reproducibility are specifics related to the use of anaesthetics. **Table 1** shows articles in the Pubmed database, published over the past year and identifies the four agents that predominate in these articles.

**Table 2** shows our audits of anaesthetics used in papers published in *BJP* and highlights many of the changes and advances regarding accepted best practice over the past 30 years. During our audits we discovered issues related to the use of inappropriate practices and insufficient detail regarding the use of anaesthetics in articles published in *BJP*. We found substantial divergence in the details that authors provide regarding dose, flow rates for volatiles agents, and the route of administration of agents. There is typically no data provided on maintenance doses used for agents that are employed nor information regarding survival or long-term procedures. The audit also identified the use of procedures that should require anaesthesia but that lack such information. Many studies do not identify methods (agent, dose, time) for killing, or if an anaesthetic agent (or $CO_2$) is used, a secondary method (e.g., decapitation, exsanguination) to ensure death.

The noted variability and absence in detail of anaesthetic management during animal experimentation in manuscripts published in *BJP* indicates a pressing need for guidance regarding administration of anaesthetics. Appropriate study design dictates a proper choice of anaesthetic agents. Importantly, anaesthetic agents have a profound effect on animal physiology, including, but not limited to, changes in cerebral blood flow and metabolism, cardiac function, blood pressure, respiratory rate and tidal volumes. Thus, the selection of an anaesthetic is an important aspect of study design and can impact experimental outcomes. Furthermore, humane treatment of animals during research studies is paramount and is related to how animals are anaesthetized. In addition, the method of killing can profoundly

impact upon the morphology and physiology of samples that are collected and subsequently assessed. Our audit demonstrates that many authors forget to provide these details. As *BJP* Editors, we are not insisting upon their inclusion as they may not necessarily change the outcomes or interpretation of a study. However, anaesthetics can impact on many outcome measures, and will almost certainly influence the reproducibility of the findings.

The choice of an anaesthetic agent affects the survivability of animals and their overall comfort. Anaesthetics that are suitable for general anaesthesia in surgical procedures might not be suitable analgesics for sedation in non-surgical procedures. **Table 3** provides a list of e anaesthetics currently deemed suitable for short-term and long-term anaesthesia in rodents (P. Flecknell, 2009; Mazze, Rice, & Baden, 1985). **Table 4** lists agents (many of which were identified in our review of *BJP* papers since 2015), that should be avoided or that raise concerns about animal welfare.

The appropriate choice of anaesthetics should be based on the level of sedation required and the anticipated effects of the anaesthetic agent on the animals under study. Responses to various anaesthetics can differ, depending on the type, strain, sex, and age of animals and by the environment in which they reside. These details must be provided within the methods of a manuscript, so that readers can consider potential impact of the approach upon measured outcomes and potentially reproduce the studies. Indeed, these details are listed within the 6-point animal experimentation guidelines for *BJP* as points 1 and 4 (Lilley et al., 2020) respectively (also points 8 and 15 of the ARRIVE 2.0 guidelines (Percie du Sert et al., 2020)).

How does one choose the correct anaesthetic agent? This is not a simple task since anaesthetics exert numerous effects (sometimes species-specific) on various organ systems. The choice of anaesthetics is particularly important in studies of outcomes of a particular disease model. Below we summarise current views regarding drugs that are commonly used as anaesthetics, their pharmacological targets, effects and side-effects. We also define the expectations and guidelines regarding the details required for anaesthetic use in manuscripts published in *BJP*.

## Inhalation (volatile) anaesthetics

Inhalational anaesthetics are commonly used to provide general anaesthesia for both small and large animals. Since their concentrations in sampled gas can be measured continuously, they afford the advantage of rapid and predictable titration of anaesthetic depth and ease of continual adjustment. Volatile anaesthetics commonly used for animal anaesthesia include isoflurane, sevoflurane and halothane (but the use of halothane is now widely deprecated due to issues discussed below). Diethyl ether, the first widely used inhalational agent, is no longer recommended because it is highly flammable at therapeutic concentrations and is a profound respiratory irritant.

As in humans, the effective dose and potency of inhalational agents is measured as the minimum alveolar concentration (MAC). MAC is the effective dose in which 50% of animals do not respond to a noxious stimulus (Tyler, 2013). The MAC value for a given agent is consistent across a wide range of animal species and is even more consistent within species.

MAC is affected by circadian rhythms, such that MAC values are higher during periods when the animals are normally highly active (Quasha, Eger, & Tinker, 1980). In addition, MAC values decrease with reductions in core temperature of the animal. For example, halothane requirements are reduced by 5% for degree Centigrade drop in core temperature (Liu, Hu, & Liu, 2001). As in humans, MAC values in experimental animals are reduced with increasing age. To enable reproducibility it is thus essential that authors detail the age of animals and how their body temperature was maintained.

All volatile anaesthetics dose-dependently reduce systemic vascular resistance and mean arterial pressure (MAP). At clinically relevant concentrations, this reduction is modest. However, at increased doses, the reduction in systemic vascular resistance, coupled with a decrease in myocardial contractility may reduce cardiac output and alter regional blood flow and its distribution. Isoflurane is commonly used for anaesthesia in coronary perfusion studies, introducing a potential confounder. However, isoflurane maintains cardiovascular function better than enflurane and halothane. The cardiac anaesthetic index—the ratio of the concentration that produces cardiovascular collapse to anaesthetic dose—is higher for isoflurane than for halothane (5.7 vs. 3.0, respectively) (Wolfson, Hetrick, Lake, & Siker, 1978).

The reduction in cardiac output caused by the volatile anaesthetics also reduces renal blood flow (RBF) and glomerular filtration rate (GFR). These effects are dose-dependent and may transiently increase plasma concentration of urea, nitrogen and creatinine during anaesthesia. Accordingly, healthy animals may produce a smaller volume of urine while anesthetized;, such confounders should be considered in designing anesthetic choice in renal studies. In addition, the vasodilatory effects of volatile anaesthetics can increase in cerebral blood flow. Isoflurane differs from halothane and enflurane, in that the increased cerebral blood flow associated with isoflurane can be offset by hyperventilation (Boarini, Kassell, Coester, Butler, & Sokoll, 1984). Because of this, isoflurane is the inhalational anaesthetic of choice for experiments that cannot tolerate increases in cerebral blood flow.

Volatile anaesthetics can depress hepatic function and may cause hepatocellular damage. The predominant mechanism of this effect is via reduced hepatic blood flow and oxygen delivery (Vollmar et al., 1992). Of all the commonly used volatile anaesthetics in animals, isoflurane is regarded as the best for maintaining hepatic oxygen delivery, while halothane produces the most hepatotoxicity (via metabolites), limiting its use as an experimental and clinical agent.

**Injectable Sedatives**

Injectable sedatives produce dose-dependent sedation but lack the efficacy to provide complete loss of consciousness and responsiveness to noxious stimuli. Therefore, these agents are generally used only for sedation and tranquilization and are administered as adjuncts to general anaesthetics. They should ***not*** be used by themselves to provide general anaesthesia. Classes of drugs in this category include dopamine receptor antagonists, $\alpha_2$-adrenoceptor agonists and $GABA_A$ receptor-positive allosteric modulators (PAMs) $GABA_A$ receptors.

**$\alpha_2$-Adrenoceptor agonists**

$\alpha_2$-Adrenoceptor agonists provide dose-dependent sedation and analgesia via stimulation of CNS $\alpha_2$-adrenoceptors. Their effects are dose-dependent, and these compounds are administered for sedation/analgesia but as noted above, ***not*** as anaesthetics. Commonly used agents in this class include xylazine, detomidine, dexmedetomidine, medetomidine and romifidine. There is marked variation in sensitivity to these drugs among animal species (England & Clarke, 1996; Torneke, Bergstrom, & Neil, 2003). Agents in this class have a rapid absorption following intramuscular or subcutaneous injection. Xylazine is rapidly metabolized, while redistribution accounts for most of the termination of effect for detomidine and medetomidine. Xylazine is commonly used for *in vivo* experiments published in *BJP*, such as those using intravital microscopy to visualize leukocyte recruitment (Young, Voisin, Wang, Dangerfield, & Nourshargh, 2007) and is often administered in combination with ketamine. Xylazine can cause nausea/vomiting after its administration--an adverse effect that does not occur in rodents since they lack the ability to vomit.

All $\alpha_2$-adrenoceptor agonists produce dose-dependent reduction in BP and bradycardia (J. M. Savola, 1986; J-M Savola, Ruskoaho, Puurunen, Salonen, & Kärki, 1986; Yamashita et al., 2000). Cardiac arrhythmias, including heart AV block, have been described following administration of these drugs (Vainio, 1989). Cardiac monitoring should be employed when these drugs are used; their use may be relatively contraindicated if cardiac function must be preserved during experimentation.

Dexmedetomidine, detomidine and medetomidine are derivatives of imidazoles that inhibit steroidogenesis. These compounds blunt the cortisol response to ACTH,, and inhibit aldosterone, corticosterone and cortisol release. There is between-species variability in the blunting of this stress response (Maze et al., 1991; Raekallio, Vainio, & Scheinin, 1991; Schoemaker, Mol, Lumeij, Thijssen, & Rijnberk, 2003; Venn, Bryant, Hall, & Grounds, 2001). Researchers should consider this aspect of their activity and whether such responses are pertinent to the study being conducted.

**Dopamine receptor antagonists**

Dopamine receptor antagonists are a class of sedative agent. Sedation occurs at low doses (<0.05-0.5 mg/kg for most drugs). At higher doses, the sedative effect is prolonged and dystonic reactions are more prevalent. The two major groups of these agents are phenothiazines and butyrophenones.

Currently used phenothiazine derivatives include chlorpromazine, promazine, and acepromazine. Phenothiazines can lower the seizure threshold; thus, these agents, particularly acepromazine, are relatively contraindicated for use in animals with a history of epilepsy. However, there is little evidence to suggest that these agents potentiate seizure activity in animals (Tobias, Marioni-Henry, & Wagner, 2006). Hypotension can occur due to direct myocardial depression and venodilatation. Acepromazine, in particular, disrupts platelet function in rats and dogs (Barr, Ludders, Looney, Gleed, & Erb, 1992; Dwyer & Meyers, 1986) and impacts on models assessing platelet reactivity. In addition, acepromazine is contraindicated in rabbits due to myositis and paralysis (Bree, Cohen, & Abrams, 1971).

Butyrophenones include droperidol, azaperone and fluanisone, and are agents commonly used to treat schizophrenia. Droperidol, is also used to treat nausea, has received a black box warning by the FDA for QT prolongation, and is reported to inhibit cardiovascular sodium, calcium and potassium channels in cardiovascular tissues(Pacher & Kecskemeti, 2004). Its sedative potency exceeds that of chlorpromazine. Droperidol and azaperone have protective effects against traumatic shock (Niemegeers, Van Nueten, & Janssen, 1974). There is controversy regarding antinociceptive effects of the butyrophenone compounds, but evidence suggests an analgesic effect and potentiation of opioid-induced analgesia (Greene, 1972; Kyles, Waterman, Livingston, & Vetmed, 1993; Olson & Renchko, 1988).

## Injectable Hypnotics

Injectable hypnotics include drugs or classes of drugs that, by themselves, will cause a state of unconsciousness and immobility, often referred to as a state of general anaesthesia. Numerous classes of drugs fall into this category but two classes are the most commonly used in manuscripts published in *BJP*: gamma aminobutyric acid (GABA) agonists and N-methyl-D-aspartate (NMDA) antagonists.

### $GABA_A$ Receptor Positive Allosteric Modulators (PAM)

Chemical classes acting through $GABA_A$ receptors include the barbiturates, etomidate/medetomidate, and propofol. All of these compounds activate neuronal $GABA_A$ receptors, thereby increasing chloride ion conductance and eliciting hyperpolarization. Agents that act via $GABA_A$ receptors are highly potent CNS depressants and will result in unconsciousness and immobility. However, these agents lack antinociceptive effects and are often co-administered with an analgesic.

*Barbiturates*

Barbiturates, a class of $GABA_A$ receptor PAMs, are derived from barbituric acid and have variable potency. Drugs in this class include: thiopental, thiamylal, pentobarbital and methohexital. These drugs used to be classified as long, short, and ultra-short acting agents. However, this classification is no longer used because repeated or continuous administration of barbiturates results in drug accumulation and prolonged clinical effect. Solutions of this class of drugs are alkaline (pKa 7.5-7.8), incompatible with acidic drugs, and can precipitate when co-administered with opioids and neuromuscular blocking agents. To prevent this, intravenous lines should be flushed when these agents are used concomitantly.

As a chemical class, barbiturates decrease cerebral blood flow and, therefore, reduce intracranial pressure. In addition, they also reduce cerebral metabolic rate and may be neuroprotective. However, these agents do not protect against focal neurologic ischaemic events (Hall & Murdoch, 1990; Murdoch & Hall, 1990). Potential protective effects should thus be considered in studies involving neuronal endpoints.

Barbiturates reduce systemic vascular resistance and can reduce myocardial contractility (Stowe, Bosnjak, & Kampine, 1992). However, barbiturates preserve the cardioprotective effects of ischaemic preconditioning and, therefore, may be useful when anaesthesia is

required in such experiments (Mullenheim, Molojavyi, Preckel, Thamer, & Schlack, 2001). Changes in heart rate are species-dependent: Barbiturates increase heart rate in dogs but not in rats and rabbits (P. A. Flecknell, John, Mitchell, Shurey, & Simpkin, 1983; Manders & Vatner, 1976; Wixson, White, Hughes, Lang, & Marshall, 1987).

There is considerable between-strain and within-strain variability in the dose-response relationship of barbiturates. The variation in sleep times varies among different strains of mice, while historical evidence showed that female rats take three times longer to recover from barbiturate-induced hypnosis than did males (Holck, Kanan, Mills, & Smith, 1937; Lovell, 1986a, 1986b, 1986c). Due to the necessity to include both sexes in experimental studies (Docherty et al., 2019), this is an important consideration for in vivo experiments.

*Etomidate and Metomidate*

Etomidate and metomidate are imidazole derivatives acting as PAMs to activate $GABA_A$ receptor. They induce hypnosis and general anaesthesia although, by themselves, lack significant analgesic effects. Etomidate and metomidate minimally suppress myocardial contractility and have little effect on systemic vascular resistance (Pascoe, 1992). These drugs may thus be useful for experiments in which perturbations in myocardial function and/or BP are to be avoided.

Side-effects of these agents include injection site pain, nausea and myoclonic seizures. Both etomidate and metomidate inhibit adrenal steroidogenesis by decreasing 11-β-hydroxylase activity (Mitterhauser et al., 2003). Adrenocortical suppress can last 2-6 hours in dogs and at least 5 hours in cats after a single bolus (Dodam, Kruse-Elliott, Aucoin, & Swanson, 1990; Moon, 1997). Septic shock can occur in bacteraemic animals given these agents.

*Propofol*

Propofol is a potent $GABA_A$ receptor PAM used for induction and maintenance of anaesthesia. The drug itself is insoluble in aqueous solution and therefore is formulated in an emulsion usually containing soybean oil, glycerol and egg phosphatides. These formulations, particularly the soybean oil, support bacterial growth. Hence, aseptic techniques are required during the handling, preparation and administration of propofol. Propofol is associated with injection site pain; however, this can be relieved by injection in a large vein. Since pain is a response that can impact on multiple functions, detail regarding how this drug is administered is critical in understanding and accounting for potential impact upon experimental findings. Anaphylactoid reactions have been described in rats and pigs (Glen & Hunter, 1984).

Propofol is similar to barbiturates, in that the recovery from anaesthesia is rapid following a single bolus, owing to its extensive redistribution and clearance from the plasma. Propofol can be administered as a continuous infusion for maintenance of anaesthesia as there is little accumulation of drug. Hence the half-life of response is relatively short and independent of infusion duration. Despite the short action and relatively rapid recovery, the latter is not necessarily faster than volatile anesthetics. Sensitivity to propofol may be affected by age, with younger animals requiring higher doses for induction of anaesthesia. Older animals have higher CNS concentrations of propofol (Larsson & Wahlstrom, 1998).

Common side-effects of propofol include a dose-dependent decrease in mean arterial BP (Blake, Jover, & McGrath, 1988; Carmichael, Crawford, Khayyam, & Saldivia, 1993; Ilkiw, 1992). Administration in animals that are hemodynamically compromised can be harmful (Ilkiw, Pascoe, Haskins, & Patz, 1992). Propofol decreases cerebral oxygen consumption, intracranial pressure and cerebral blood flow (Marik, 2004). It is also a potent anti-convulsant (Lee, Moscicki, & DiFazio, 1998; Marik, 2004). Propofol can cause significant respiratory depression at doses necessary to induce hypnosis. The magnitude of respiratory depression seems to be species-dependent (Bellman & Pleuvry, 1981; Glen, 1980; Matthews, Brown, Barling, Lovering, & Herrig, 2004).

**NMDA Antagonists**

*Ketamine*

Ketamine is a potent N-methyl-D-aspartate (NMDA) antagonist. It is a derivative of phencyclidine and has dissociative anaesthetic effects, believed to be secondary to the inhibition of thalamocortical pathways and stimulation of the limbic system. Unlike other injectable hypnotics, general anesthesia with ketamine results in less attenuation of ocular and pharyngeal reflexes. Ketamine is unique among injectable anesthetics in that it inhibits postsynaptic uptake of catecholamines, thereby preserving sympathetic tone.

Ketamine is water-soluble, and exists as a racemic mixture of R(-) and S(+) isomers, each exhibiting varying levels of anesthetic potency (Muir & Hubbell, 1988). Ketamine has a rapid onset of action, with peak CNS concentrations occurring within one minute after intravenous injection and a rapid return of consciousness secondary to the drug's distribution from the CNS to other tissues. Ketamine can be given intravenously, intramuscularly, or intraperitoneally. However, the intraperitoneal administration of ketamine should be avoided in small animals and rodents as it may cause discomfort (P. Flecknell, 2009). The drug is frequently administered in combination with xylazine, a potent $\alpha_2$ adrenoceptor agonist.

Ketamine increases sympathetic nervous system activity. After administration, myocardial contractility and heart rate are increased. These effects can be nullified by concomitant inhalational anesthesia, ganglionic blockade, epidural anesthesia, or spinal cord transection. The effects of ketamine on MAP are variable and species-dependent. Ketamine increases MAP in rats while decreases in MAP have been demonstrated in rabbits. The effects of ketamine on BP in non-human primates is insignificant (Reutlinger, Karl, Vinal, & Nieser, 1980).

Unlike other intravenous hypnotics, ketamine preserves protective airway reflexes. In addition, the sympathomimetic effects of ketamine promote smooth muscle relaxation in the bronchial tree. Ketamine also has a minimal effect on oxygenation and ventilation. However, concurrent administration of other anesthetic agents may override ketamine's modest effect.

Ketamine increases cerebral perfusion and intracranial pressure. Prior evidence suggested that ketamine induces seizures in dogs and cats (Wright, 1982). However, ketamine raises the seizure threshold in rats and mice, and does not alter seizure thresholds in epileptic animals.

Although myoclonus and seizure-like activity can be observed following its administration, EEG evidence of epilepsy has not been demonstrated and as such, ketamine is considered to have anticonvulsant effects (Modica, Tempelhoff, & White, 1990).

**Guidelines to be considered**

The availability and adoption of anaesthetic agents in scientific experimentation varies throughout the world. However, it is important to adopt uniform standards that eventually, as they become widely adopted, will ensure limited pain, suffering, distress, or harm to experimental animals, whilst simultaneously leading to improved reproducibility and potentially translatability. In setting these standards we expect thatdeviations from the standards should be justified scientifically. The key guidance we suggest is as the five points listed below:

1. **Anaesthetic details and dosing:** Dose/concentration, route of administration, vehicle or flow rate, and maintenance dose, if any used, of anaesthetics for various procedures must be provided.
2. **Methods for monitoring of state of anaesthesia:** In general, methods should describe the procedures used to monitor and respond to changes in the state of anaesthesia and the general well-being of the animal during the procedures being undertaken. The depth of anaesthesia, assessed by a mild noxious stimulus (i.e., paw pinch and frequency of assessment), and additional maintenance doses of anaesthetic administered should be reported.
3. **Monitoring of physiological parameters:** Physiological parameters and vital signs during anaesthesia should be monitored and reported such as body temperature, heart rate, respiratory rate and effort (visual, oxygenation assessment), MAP, pain and distress should be carried out and reported.
4. **Anaesthetic choice relevant for species and study design:** Consideration for anaesthetic choice changes with studies involving terminal versus survival procedures and these should be clearly outlined in the methods.
5. **Method of killing:** where anaesthetic overdose is used full details must be provided including the method for confirmation of death.

**Concluding thoughts**

The *British Journal of Pharmacology* aims to set rigorous standards for animal experimentation, including with respect to anaesthetics used, in the manuscripts published in the *Journal*. The guidance outlined above, regarding the reporting of anaesthetic procedures, will be adopted immediately and the use of optimal anaesthetic agents will be required by 2024. With the many choices of agents available, authors must consider the experimental approach, specific monitoring and the characteristics of the animal species being used when considering the most appropriate anaesthetic for studies in order to optimize scientific integrity and animal ethics.

**Table 1: Anaesthetics usage in rats (2021-22).** The results of PubMed Central searches of full-text articles in the last year using the search term "*anaesthesia rat y*", where "*y*" is each of the above-named anaesthetics searched.
[1] The number of articles using ether is an estimate, based on sampling of 50 articles, where 38% of hits mentioned ether used as an anaesthetic (search performed 29th August 2022).

| **Agent** | **Anaesthesia (rat) in last year (no. of articles)** | **% of total** |
|---|---|---|
| Isoflurane and other similar agents | 7,107 | 44.9% |
| Ketamine<br>(of which ketamine + xylazine or + dexmedetomidine) | 3260<br>(2362) | 20.6% |
| Pentobarbitone<br>Thiopental | 2630<br>265 | 16.6%<br>1.7% |
| Propofol | 1142 | 7.2% |
| Ether[1] | 326 (estimate) | 2.1% |
| Chloral hydrate | 468 | 3.0% |
| Urethane | 450 | 2.8% |
| Tribromoethanol | 96 | 0.6% |
| Chloralose | 66 | 0.4% |
| Alphaxalone | 20 | 0.1% |

**Table 2:** Audit of original articles published between 2015-2022 in *BJP*. Audits were conducted on original articles in issue 1 and 14 for each chosen year. Where less than 10 articles were available, then issues following these were taken to provide enough articles with 10 as a minimum for each year.

| Year | Issue | No Original Papers sampled | % Species used stated | %Anaesthetic appropriate for purpose | %Anaesthetic appropriate for species | | %Dose used given | %Route of administration given | %Details of maintenance doses given | %Euthanasia method stated | %Confirmation of death |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Recovery | Killed/euthanasia | | | | | |
| 2015 | 1,14 | 12 | 100 | 60 | 86 | 25 | 75 | 92 | 17 | 25 | 0 |
| 2016 | 1,14 | 20 | 90 | 60 | 40 | 44 | 35 | 35 | 5 | 45 | 0 |
| 2019 | 3,5,6,14,15 | 18 | 100 | 67 | 77 | 50 | 67 | 72 | 22 | 50 | 0 |
| 2020 | 1,14 | 15 | 100 | 82 | 57 | 40 | 40 | 53 | 7 | 47 | 0 |
| 2022 | 1,14 | 11 | 100 | 82 | 75 | 91 | 46 | 82 | 0 | 82 | 9 |

| Standard of reporting | | |
|---|---|---|
| | | |
| Poor | Better | Good |
| 0-49 | 50-75 | 76-100 |

**Table 3: Common anaesthetics and doses used in mice and rats.**

| | Mouse | Rat |
|---|---|---|
| **Inhaled Anaesthetics** (MAC values listed) | | |
| Isoflurane | 1.34 | 1.46 |
| Sevoflurane | 2.5 | 2.7 |
| **Injectable Anaesthetics and Possible Combinations** (mg/kg value) | | |
| Ketamine HCL (deep sedation, mild to mod analgesia) | 100-200 (I.M., I.P.) | 50-100 (I.M., I.P.) |
| Pentobarbital (light anaesthesia for <60min) | 40-50 (I.P.) | 40-50 (I.P.) |
| Propofol (surgical anesthesia for 5-10 minutes) | 26 (I.V.) | 10 (I.V.) |
| Thiopental (surgical anesthesia for 5-10 minutes) | 30-40 (I.V.) | 30 (I.V.) |
| Tribromoethanol (surgical anesthesia for 15-45 minutes) | 240 (I.P.) | |
| Ketamine/Acepromazine (light anesthesia <30 minutes) | 100/5.0 (I.P.) | 75/2.5 (I.P.) |
| Ketamine/Acepromazine/ Xylazine (surgical anesthesia ~30 minutes) | 80-100/ 3.0/10 (I.P.) | 40-50/0.75/2.5 (I.M., I.P.) |
| Ketamine/Diazepam (Immobilization, light anesthesia for <30 minutes) | 100/5.0 (I.P.) | 75/5.0 (I.P.) |
| Ketamine/Medetomidine ((Surgical anesthesia for <30 minutes) | 75/1.0 (I.P.) | 75/0.5 (I.P.) |
| Ketamine/Midazolam (Immobilization, light anesthesia for <30 minutes) | 100/5.0 (I.P.) | 75/5.0 (I.P.) |
| Ketamine/Xylazine (Surgical anesthesia for <30 minutes) | 80-100/10 (I.P.) | 75-100/10 (I.P.) |

**Table 4: List of agents that should be avoided or that raise concerns about animal welfare**

| DRUG | RATIONALE |
|---|---|
| Chloroform | Broncho-irritant; can cause hepatotoxicity; carcinogenic |
| $CO_2$[1] | Has no anaesthetic properties; can cause asphyxiation. |
| Diethyl ether | Broncho-irritant; highly flammable |
| Halothane | Can cause hepatotoxicity |
| Chloral hydrate | Can cause significant respiratory depression; efficacy as an anaesthetic questionable in small animals |
| Urethane[2] | Carcinogenic; suitable only for non-recovery procedures |

Use of above agents as anaesthetics in animal experiments in manuscripts submitted to the BJP will be a reason for **rejection of the Manuscript** by the Senior Editors.

[1] $CO_2$ can be used for humane killing when used in combination with a secondary method to confirm death (i.e., exsanguination, decapitation, cervical dislocation).

[2] There is potential to use urethane for select procedures, particularly for CNS studies, due to its actions. The nature of the studies would need to be described in detail, and the use of urethane justified.

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## Hyperlinks

isoflurane https://www.guidetopharmacology.org/GRAC/LigandDisplayForward?ligandId=2505

sevoflurane https://www.guidetopharmacology.org/GRAC/LigandDisplayForward?ligandId=7296

halothane https://www.guidetopharmacology.org/GRAC/LigandDisplayForward?ligandId=2401

xylazine https://www.guidetopharmacology.org/GRAC/LigandDisplayForward?ligandId=523

dexmedetomidine https://www.guidetopharmacology.org/GRAC/LigandDisplayForward?ligandId=521

chlorpromazine https://www.guidetopharmacology.org/GRAC/LigandDisplayForward?ligandId=83

promazine https://www.guidetopharmacology.org/GRAC/LigandDisplayForward?ligandId=7281

droperidol https://www.guidetopharmacology.org/GRAC/LigandDisplayForward?ligandId=7172

fentanyl https://www.guidetopharmacology.org/GRAC/LigandDisplayForward?ligandId=1626

GABA https://www.guidetopharmacology.org/GRAC/LigandDisplayForward?ligandId=1067

NMDA https://www.guidetopharmacology.org/GRAC/LigandDisplayForward?ligandId=4268

propofol https://www.guidetopharmacology.org/GRAC/LigandDisplayForward?ligandId=5464

etomidate https://www.guidetopharmacology.org/GRAC/LigandDisplayForward?ligandId=5463

thiopental https://www.guidetopharmacology.org/GRAC/LigandDisplayForward?ligandId=2579

thiamylal https://www.guidetopharmacology.org/GRAC/LigandDisplayForward?ligandId=7305

pentobarbital https://www.guidetopharmacology.org/GRAC/LigandDisplayForward?ligandId=5480

methohexital https://www.guidetopharmacology.org/GRAC/LigandDisplayForward?ligandId=7233

enflurane https://www.guidetopharmacology.org/GRAC/LigandDisplayForward?ligandId=7175

dopamine receptors https://www.guidetopharmacology.org/GRAC/FamilyDisplayForward?familyId=20

adrenoceptors https://www.guidetopharmacology.org/GRAC/FamilyDisplayForward?familyId=4

$GABA_A$ receptors https://www.guidetopharmacology.org/GRAC/FamilyDisplayForward?familyId=72

ketamine https://www.guidetopharmacology.org/GRAC/LigandDisplayForward?ligandId=4233

cortisol https://www.guidetopharmacology.org/GRAC/LigandDisplayForward?ligandId=2868

ACTH https://www.guidetopharmacology.org/GRAC/LigandDisplayForward?ligandId=3633

aldosterone https://www.guidetopharmacology.org/GRAC/LigandDisplayForward?ligandId=2872

corticosterone https://www.guidetopharmacology.org/GRAC/LigandDisplayForward?ligandId=2869

11β-hydroxylase https://www.guidetopharmacology.org/GRAC/ObjectDisplayForward?objectId=1359

phencyclidine https://www.guidetopharmacology.org/GRAC/LigandDisplayForward?ligandId=4282

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