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DIRECT NOSE-TO-BRAIN TRANSPORT IN CENTRAL NERVOUS SYSTEM DISORDERS: APPRAISING THE QUALITY OF PRECLINICAL EVIDENCE AND ESTABLISHING STANDARDS FOR ITS REPORTING

Jyoti Pandit*[1]

Sunaina[2]

[1]*Assistant Professor, School of Pharmaceutical Sciences, Jigyasa University (Formerly Himgiri Zee University), Dehradun, Uttarakhand*

[2]*Student, B Pharm 2nd Year, School of Pharmaceutical Sciences, Jigyasa University (Formerly Himgiri Zee University), Dehradun, Uttarakhand*

Corresponding author:
Jyoti Pandit

Email: jyotipandit082002@gmail.com

ABSTRACT

The proposition that a drug placed in the nasal cavity can reach the central nervous system without first entering the systemic circulation has organised two decades of preclinical pharmaceutics, yet the claim remains contested at its foundation. This review argues that the difficulty is not biological implausibility. Anatomical substrates for olfactory, trigeminal and perivascular transfer are real and have been characterised in detail. The difficulty is evidentiary. Direct transport is a claim about route, whereas nearly every experiment that supports it measures only outcome, namely the amount of drug recovered from brain tissue. Bridging that gap requires a specific inferential architecture, and the metrics the field relies upon, drug targeting efficiency and direct transport percentage, are ratio estimators whose validity depends on assumptions about dose equivalence, systemic disposition, sampling adequacy and tissue integrity that most published designs neither satisfy nor examine. The steps that most strongly determine the result, namely instillation volume and depth, animal posture, anaesthesia, the intravenous comparator and the dissection protocol, are also the steps least completely reported. Pooled surveys of this literature have repeatedly found variability in reported targeting that does not track the physicochemical properties of the delivered agent, which is the signature of methodological rather than pharmaceutical variance. Generic reporting frameworks, ARRIVE 2.0, SYRCLE and MIRIBEL, each address part of the problem and none addresses the route-specific part. This review therefore sets out a reporting standard built for intranasal central nervous system studies, organised across administration, comparator, sampling, analysis and translation, together with a four-tier scheme that states plainly which conclusions a given design can carry. Adopting an explicit evidential grammar would allow the field to accumulate evidence rather than merely accumulate publications.

Keywords: Nasal administration; Drug delivery systems; Blood-brain barrier; Central nervous system agents; Research design; Guidelines as topic; Reproducibility of results.

1. Introduction

Few ideas in drug delivery have the immediate intuitive appeal of the nose-to-brain hypothesis. The olfactory mucosa is the one site in the body where neurons of the central nervous system extend to an external epithelial surface, separated from the outside world by little more than a layer of mucus and the supporting cells that surround them [1,2]. If a molecule could be persuaded to travel that path, it would enter the brain without negotiating the blood-brain barrier, without first being diluted into the whole body, and without a needle. For disorders whose pharmacology has been frustrated for decades by poor central exposure, the attraction of such a route is obvious and entirely legitimate.

The idea is also old enough to have been tested thoroughly. Reviews of transport from the nasal cavity to the central nervous system were already synthesising a substantial body of tracer and pharmacokinetic work at the turn of the century [3]. In the two decades since, the field has generated a continuous stream of nanoparticulate carriers, mucoadhesive gels, permeation enhancers and targeting ligands, each evaluated against the same general question of whether brain exposure after nasal dosing exceeds what systemic absorption alone would predict.

What has not grown at the same rate is confidence in the answer. In 2007 a critical appraisal of approximately one hundred published studies concluded that only a small minority employed designs capable of distinguishing direct transport from ordinary systemic delivery, and that no pharmacokinetic evidence then available supported the claim of enhanced delivery to brain targets in humans [4]. That paper was widely cited and, in a certain sense, widely ignored: it was absorbed into introductions as a gesture towards controversy rather than taken up as a specification for better experiments. Nearly twenty years later, a survey of the clinical literature reached a structurally identical conclusion, namely that pharmacokinetic and clinical observations following intranasal dosing are consistent with the feasibility of the route but do not constitute evidence that delivery occurred predominantly by olfactory or trigeminal pathways [5]. A controversy that survives two decades of intensive investigation without resolution is rarely a controversy about facts. It is usually a controversy about what would count as a fact.

That is the position taken here. The proposition of this review is that the central weakness of the preclinical nose-to-brain literature is neither biological nor technical but evidentiary, and that it is therefore correctable by changes in design and reporting rather than by further formulation innovation. Three observations support the diagnosis.

First, direct transport is a claim about route, while almost every experiment conducted to support it measures outcome. Finding drug in brain tissue after nasal administration establishes that the drug reached the brain. It says nothing by itself about the path taken, because the systemic circulation offers a complete and sufficient alternative explanation for the same observation. Converting an outcome measurement into a route

inference requires additional structure in the experiment, and that structure is frequently absent.

Second, the quantitative instruments the field has adopted to perform that conversion, principally drug targeting efficiency and direct transport percentage, are ratio estimators resting on a chain of assumptions that are seldom stated and almost never tested. When the assumptions fail, the metrics do not fail loudly. They return a number, and the number is reported.

Third, the experimental steps that most powerfully determine the value of those metrics are the steps least completely described in published reports. How much liquid was instilled, how deeply, with the animal in what position and under what anaesthetic, against which systemic comparator at which dose, and with brain tissue separated from the dosing site by what dissection protocol: these details decide the answer, and their omission makes a study uninterpretable rather than merely incomplete.

The consequence is a literature that accumulates publications faster than it accumulates evidence. Pooled analyses of this body of work have repeatedly encountered a telling pattern, namely that the spread in reported targeting values does not correlate with the physicochemical characteristics of the compounds or carriers being delivered [6,7]. Variance that does not track the independent variable is not biological variance. It is methodological noise, and no amount of additional formulation chemistry will reduce it.

This review does not report new experimental data and does not attempt to adjudicate whether direct nose-to-brain transport occurs. The mechanistic literature makes clear that transfer along perineural and perivascular routes is anatomically available and demonstrable under controlled conditions [1,8]. The question addressed here is narrower and more tractable: what must a preclinical study do, and what must it report, before its results can bear the weight of a direct-transport claim? Sections 3 to 5 set out what such a claim asserts, examine the metrics used to support it and catalogue the recurrent design failures. Section 6 explains why the existing generic reporting frameworks, valuable as they are, leave the route-specific problem untouched, and Section 7 proposes a reporting standard and an evidence-grading scheme built for this route. The argument throughout is that the field does not need a moratorium on nose-to-brain research. It needs an explicit evidential grammar, and journals and reviewers willing to apply one.

2. Scope and approach

This is a narrative, methodologically focused review rather than a systematic review, and it is labelled as such deliberately: the object of appraisal is the inferential structure of a research literature, which is not a question that a pooled effect estimate can answer. The search nevertheless followed a structured protocol so that the evidence base is traceable.

MEDLINE via PubMed, Europe PMC and Scopus were searched from database inception to September 2026, combining terms for the route

(intranasal, nose-to-brain, nasal administration, olfactory, trigeminal) with terms for the target (brain, central nervous system, cerebrospinal fluid) and with terms for the methodological object of interest (drug targeting efficiency, direct transport percentage, study design, reporting quality, risk of bias, reproducibility, translation). Reference lists of retrieved reviews and critiques were screened for additional sources, and reporting frameworks were retrieved from their originating publications and official guideline documents. Three categories of source were prioritised: critical appraisals and pooled analyses of the nose-to-brain literature, comparative anatomical and physiological descriptions of the nasal cavity and its central connections, and established reporting or risk-of-bias frameworks from adjacent fields. Individual formulation studies were consulted to characterise prevailing practice, not to extract or compare their findings.

Two boundaries are stated explicitly. No primary experimental results from human participants or from laboratory animals are reported in this article. Where a study is cited, it is cited for its methodological content, for its description of anatomy or physiology, or for the design practice it exemplifies, and effect estimates from such studies are not reproduced. Comparative anatomical and physiological measurements are retained, because the argument about interspecies scaling cannot be made without them. Second, the review does not present a pooled quantitative synthesis. The published studies measure heterogeneous endpoints under heterogeneous administration conditions, and the case made in Sections 4 and 5 is precisely that these values are not commensurable. Pooling them would contradict the argument of the paper.

3. What a direct-transport claim asserts

3.1 The anatomical substrate

Three routes from the nasal cavity to the central nervous system are recognised, and they differ in destination, in timescale and in the evidence that supports them.

The olfactory pathway begins at the olfactory epithelium in the roof of the nasal cavity, where the dendrites of bipolar sensory neurons reach the mucosal surface and their axons pass in bundles through the perforations of the cribriform plate to terminate in the olfactory bulb [1,3]. Two modes of transfer are available along this pathway and they are frequently conflated. Intracellular transfer involves uptake into the olfactory neuron followed by axonal transport, a process whose speed is set by the machinery of the cell. Extracellular transfer moves material through the channels that surround the axon bundles and the associated olfactory ensheathing cells, and is governed by bulk flow rather than by cellular transport. The distinction has an empirical consequence that is often overlooked. Estimates of the time required for axonal transport along the relevant distance in rodents fall in the range of roughly three quarters of an hour to several hours, before endocytosis is taken into account [1]. Appearance of a tracer in central tissue within minutes of nasal dosing is therefore

evidence for the extracellular route, not merely a faster version of the intracellular one.

The trigeminal pathway carries material along branches of the trigeminal nerve innervating the respiratory and, to a lesser degree, the olfactory mucosa, entering the central nervous system near the brainstem and thereby reaching a quite different set of structures from those served by the olfactory bulb [1]. Because the trigeminally innervated territory is far larger than the olfactory patch, and far easier to reach with a conventional device, this route is of particular practical importance, and its contribution is systematically under-assessed in studies whose sampling is oriented towards the olfactory bulb.

The perivascular pathway is what makes rapid and widespread central distribution intelligible. Bulk flow within the perivascular spaces surrounding cerebral vessels contributes to the rapid central distribution of tracers after intranasal administration, giving access to central perivascular compartments that matched intravascular dosing does not reproduce, in a manner that depends on tracer size and that can be modulated by altering nasal epithelial permeability [8]. This work matters here for a reason beyond its mechanistic content. It illustrates the design a route claim actually requires: a systemic reference, a compartment-resolved readout rather than a bulk tissue concentration, and an experimental manipulation of the proposed pathway.

3.2 Route is not exposure

The logical structure of the direct-transport claim deserves to be stated plainly, because a great deal of confusion in this literature follows from leaving it implicit.

To say that a drug reaches the brain after intranasal administration is a statement about exposure. It is established by recovering the drug from central tissue or fluid, and it is not in dispute for a wide range of compounds. To say that a drug reaches the brain *directly* is a statement about route. It asserts that some portion of the recovered material arrived by a nasal-to-central pathway rather than by absorption into blood followed by distribution across the blood-brain barrier. These are different propositions, and the second does not follow from the first.

The reason it does not follow is that the systemic route is always available and always operating. The nasal mucosa is richly vascularised and absorbs many compounds efficiently. Any intranasal dose therefore produces a systemic exposure, and that systemic exposure produces a brain exposure of its own, the magnitude of which depends on the compound's blood-brain barrier permeability. A direct-transport claim is thus inherently a claim about a *residual*: the fraction of brain exposure that remains unexplained after the systemic contribution has been accounted for. Every credible measurement strategy in this field is, at bottom, an attempt to estimate that residual, and the quality of the estimate depends entirely on the quality of the systemic accounting.

This is why the intravenous comparator arm is not an optional refinement but the load-bearing element of the design. Without it there is no systemic term to subtract, and the residual is not estimable at any level of precision. A study that administers a formulation nasally, recovers drug from brain and reports a targeting advantage without a matched systemic reference has not produced weak evidence for direct transport. It has produced no evidence bearing on route at all, whatever the magnitude of the brain concentration observed.

A second consequence follows. Because the claim concerns a residual, its estimation inherits the error of both terms in the subtraction. Where the systemic contribution is large relative to the direct one, as it will be for any compound with appreciable blood-brain barrier permeability, the residual is a small difference between two larger and independently noisy quantities. Such estimators are unstable, and their instability worsens sharply as group sizes fall. The typical study in this field operates where that instability is severe.

4. The metrics and what they can support

4.1 Definitions

Two derived quantities dominate the quantitative reporting of nose-to-brain studies, and a third is used variably. Their definitions are set out in Table 1 alongside the assumptions each requires.

Drug targeting efficiency, usually reported as DTE%, is the ratio of brain to blood exposure after intranasal administration, divided by the same ratio after intravenous administration, with exposure expressed as area under the concentration-time curve [6]. Values at or above 100% are conventionally read as indicating preferential brain delivery by the nasal route. The metric is best understood as a normalised comparison of partitioning: it asks whether, for a given amount of drug in the blood, more drug appears in brain when the nasal route is used.

Direct transport percentage, reported as DTP%, attempts something more ambitious. It estimates the fraction of the brain exposure after nasal dosing that is attributable to a direct pathway, by first predicting the brain exposure expected from the observed systemic exposure, using the brain-to-plasma relationship established in the intravenous arm, and then expressing the excess as a proportion of total brain exposure [6]. DTP% is therefore an explicit implementation of the residual logic described in Section 3.2, and it is the more informative of the two metrics when its assumptions hold.

Drug targeting index, and various tissue-to-blood concentration ratios at a single sampling time, appear in parallel and are sometimes treated as interchangeable with the two metrics above [9,19]. They are not. A concentration ratio at one time point and an exposure ratio integrated over a full profile answer different questions, and can diverge sharply for compounds whose brain and plasma kinetics have different shapes.

4.2 The assumptions, and how they fail

Both principal metrics rest on a chain of assumptions, each of which is defensible in principle and frequently violated in practice.

The first assumption is that systemic disposition is independent of the route of entry. The intravenous arm is used to characterise how much brain exposure a given plasma exposure produces, and that characterisation is then applied to the nasal arm. This is valid only if the relationship between plasma and brain concentration is the same in both cases. Where the nasal formulation contains permeation enhancers, surfactants or nanoparticulate carriers that alter distribution, protein binding or efflux transporter activity after they enter the circulation, the assumption fails, and it fails in the direction that inflates the apparent direct fraction. The failure is undetectable within the standard design, because the intravenous arm is usually given the drug in simple solution rather than in the test formulation.

The second assumption is linearity. Applying a brain-to-plasma relationship measured at one systemic exposure level to a different systemic exposure level requires that the relationship be dose-independent across the range spanned. Where the intravenous and intranasal arms produce markedly different plasma concentrations, which is common because the nasal dose is often chosen for deliverability rather than for systemic equivalence, the extrapolation crosses a range over which saturable binding, saturable efflux or saturable elimination may operate.

The third assumption concerns the completeness of the exposure estimates. Both metrics are built from areas under the curve, and an area under the curve is only as good as the sampling schedule that produced it. Truncated schedules, sparse late sampling and extrapolation of a terminal phase estimated from few points introduce error into both the numerator and the denominator of a ratio of ratios, with the result that the propagated uncertainty in the final figure is substantially larger than the precision implied by reporting it to three significant figures. Studies working from rapid screening designs sometimes cannot construct areas under the curve at all, and the more careful among them decline to calculate the derived metrics for that reason [9].

The fourth assumption is that the tissue analysed is the tissue of interest and contains only what circulated to it. This is discussed in Section 5.3, because it is a sampling problem rather than a mathematical one, but it bears on the metrics directly: contamination of central tissue with material from the dosing site is arithmetically indistinguishable from direct transport within these formulae.

4.3 The choice of metric is itself a result

An observation that deserves more prominence than it has received is that the analytical choice made after the data are collected can determine the conclusion drawn from them. A careful standardised screening study reported that comparing tissue-to-blood ratios computed at the time of sacrifice against ratios computed from exposure integrals could lead to different conclusions from the same underlying measurements, and noted in addition that the diversity of dosing methods, sampled tissues and reported formulation detail across the literature undermines reproducibility [9]. The same study

documented that a minor difference in dissection technique between operators produced contamination sufficient to require exclusion of affected data, and that group sizes in the range typical of this field, combined with high variability, make screening conclusions questionable.

This is the signature of a field operating without an analytical convention. When the metric is chosen after the data are seen, and when several defensible metrics are available that may point in different directions, the published result reflects an unrecorded decision as much as it reflects the underlying biology. Pre-specification of the primary metric is the standard remedy, and it is essentially absent from this literature.

4.4 What pooled surveys reveal

The clearest evidence that the problem is methodological rather than pharmaceutical comes from attempts to synthesise the literature quantitatively.

A pooled analysis of brain delivery data drawn from 73 publications covering 82 compounds, comparing intranasal with parenteral routes, found wide inter-study variability in reported delivery efficiency that did not correlate with the physicochemical properties of the compounds, and found that particulate and gel-based systems conferred limited advantage over simple intranasal solutions [6]. The authors concluded that methodological standardisation and more complete reporting were prerequisites for progress. A later analysis assembled a structured database of 102 formulations from 64 articles and applied machine learning regression to relate nanosystem properties to the targeting metrics [7]. It encountered extreme skewness in the distribution of drug targeting efficiency values and, more tellingly, contradictory records in which nominally identical inputs were associated with divergent outputs.

Contradictory records with identical inputs are not a statistical curiosity. They are a direct measurement of unrecorded methodological variance. If two studies describe the same carrier, the same particle size and the same surface charge, and report targeting values that differ by an order of magnitude, then the variable responsible for the difference is something neither study reported. The pooled analyses cannot say what that variable is. The design literature can, and Section 5 sets out the principal candidates.

An instructive parallel exists in an adjacent field. A survey of a decade of nanoparticle delivery to solid tumours found that the median proportion of an administered dose reaching the target was well below one percent, a figure that had been obscured by a reporting culture oriented towards relative improvement rather than absolute delivery [10]. The nose-to-brain literature reports its results in the same relative idiom, expressing performance as a ratio against a comparator rather than as a fraction of the administered dose recovered at the target. Relative metrics are not illegitimate, but a field that reports only relative metrics loses the ability to ask whether the absolute quantities involved are therapeutically meaningful.

5. Recurrent failures of design and reporting

The methodological candidates identified above fall into five domains. Each is a point at which an unreported decision can change the result by more than the effect being measured.

5.1 The administration step

Nothing in a nose-to-brain experiment matters more than where the formulation actually goes, and nothing is reported more casually.

Administration volume is the clearest case. The rodent nasal cavity is small, with total cavity volumes in rats and mice measured in the low hundreds and low tens of microlitres respectively [11]. Conventional practice administers volumes in the region of tens of microlitres per nostril in rats and rather less in mice [28], which occupies a substantial fraction of the available space, and a cadaveric refinement study in rats found that volumes in the range of 35 to 50 microlitres were indicated on animal safety grounds, with only a cannula inserted to a specified depth reliably delivering material to the region of the olfactory bulbs [12]. The implication is uncomfortable for a large part of the literature. When instilled volume approaches or exceeds cavity capacity, the excess does not remain in the nose. It drains to the nasopharynx and is swallowed, or it is aspirated, and in either case the nominal dose and the delivered nasal dose diverge by an unrecorded and variable amount. Two studies using the same nominal dose and different volumes are not comparable, and neither is calibrated against the other.

Depth of instrumentation determines which epithelium is wetted. Material deposited in the anterior cavity contacts mainly respiratory and squamous epithelium, engaging the trigeminal territory and the systemic vasculature; material delivered deep into the cavity can reach the olfactory region. These are different experiments with different expected pathways, and a report that specifies neither the insertion depth nor the delivery device does not permit a reader to know which was performed.

Posture and anaesthesia modify deposition and clearance. Work in transparent nasal casts has shown that head position and dose splitting substantially change how far a sprayed liquid film translocates towards the posterior nose, with supine positioning and divided dosing producing markedly greater posterior deposition than an upright single dose [13]. In anaesthetised rodents, the same physical principles operate alongside anaesthetic effects on respiration, mucociliary activity and swallowing. A protocol that leaves the anaesthetic agent, the animal's head angle and the time held in position unstated has left out three variables that plausibly exceed the formulation effect in magnitude.

Mucociliary clearance sets the residence time within which any of this must occur. Modelling of regional clearance in the human nasal cavity gives mucus velocities averaging several millimetres per minute across most of the cavity wall, placing the olfactory region among the faster-clearing territories [14], and reviews of nasal physiology describe mucus renewal on a timescale of tens of minutes [15]. The classical

literature established clearance towards the nasopharynx as the default fate of deposited material, with the caution that improved absorption attributed to residence-time extension requires experimental support rather than assumption [16,17]. A formulation intended to prolong residence should therefore be evaluated with a residence-time readout, not solely with a distal brain concentration from which residence time is inferred.

5.2 The comparator arm

Section 3.2 established that the systemic reference is the load-bearing element. Several failures recur in its execution.

The comparator is sometimes omitted entirely, leaving the direct fraction unestimable. Where present, the doses are frequently not matched in a way that supports the extrapolation, so that the brain-to-plasma relationship measured at one exposure is applied at another. The comparator commonly receives drug in simple solution while the nasal arm receives the test formulation, which confounds the route comparison with a formulation comparison and, as noted in Section 4.2, breaks the assumption that systemic disposition is route-independent.

A distinct and more subtle omission is the absence of an intranasal solution control. When a nanoparticulate or gel formulation given nasally is compared only against an intravenous solution, any advantage observed is a composite of route effect and formulation effect, and the two cannot be separated. Establishing that a carrier improves nose-to-brain delivery requires the nasal solution arm as well, and the pooled finding that particulate and gel systems conferred limited advantage over simple nasal solutions [6] is a direct consequence of the fact that, when this arm is included, the increment often proves modest.

5.3 Tissue sampling and contamination

The dosing site and the sampling site are separated, in a rodent, by a few millimetres and a thin perforated plate. This proximity is the anatomical basis of the hypothesis under test, and it is simultaneously the field's most serious analytical hazard.

Material present at high concentration in the nasal cavity can contaminate central tissue during dissection, and the resulting signal is arithmetically indistinguishable from transported drug. The standardised screening study cited above documented precisely this, tracing an exclusion of data to a difference in skull-opening technique between operators [9]. That such an effect was detectable within a single laboratory operating a deliberately standardised protocol indicates how large it may be across a literature in which the dissection protocol is rarely described at all.

Two further sampling decisions shape the result. The first is whether the olfactory bulbs are analysed separately from the remainder of the brain. Pooling them raises the apparent concentration in a way that does not reflect delivery to the therapeutic target, since for most central indications the bulb is not the site of action [9]. Separate analysis is the minimum

requirement, and compartment-resolved sampling that distinguishes bulb, brainstem and forebrain regions is considerably more informative, because the olfactory and trigeminal pathways predict different regional patterns and the difference between them is testable.

The second is the handling of residual blood. Brain tissue retains an intravascular blood volume, and for a compound with high plasma concentration the drug contained in that residual blood can constitute a large share of the apparent tissue content. Perfusion, or at minimum a documented flushing procedure and a vascular correction, separates tissue drug from blood drug. Reports frequently state neither.

Cerebrospinal fluid sampling is often proposed as a cleaner alternative. It carries its own interpretive burden, since fluid concentration and parenchymal concentration are not equivalent and the relationship between them varies by compound, but a study that reports both is substantially more informative than one that reports either alone.

5.4 Species and scaling

The comparative anatomy of the nasal cavity places a hard limit on how far rodent results can be extrapolated, and the limit is quantitative.

In the rat, a large fraction of the nasal cavity surface is lined by olfactory epithelium, with comparative descriptions placing it at approximately half of the total surface, while in the human the olfactory region occupies a small minority of a much larger cavity [18]. Published estimates of the human fraction differ according to the method and the boundary definition used, ranging from approximately three percent by one morphometric account [18] to around ten percent in other analyses [20,28,30], and these figures should be quoted with their source rather than averaged, since they are not measuring quite the same thing. Whichever estimate is preferred, the direction and approximate magnitude of the disparity are consistent: the rodent presents an olfactory target that is proportionally far larger, and geometrically far easier to wet by instillation, than the human equivalent, which sits high in the roof of the cavity behind the nasal valve.

The consequence is that the olfactory contribution measured in a rodent cannot be scaled linearly to the human, and recent syntheses make the point explicitly, noting that the species difference bears directly on dose finding and on the evaluation of delivery devices [20]. The device dimension compounds the anatomical one. Conventional spray pumps deposit predominantly on the non-ciliated walls anterior to the nasal valve, a consequence of the mismatch between a circular plume and a convoluted airway whose primary evolutionary function is to protect the lung rather than to admit drugs [21], and only a small proportion of a conventionally sprayed dose reaches the upper nasal space where the olfactory mucosa lies [20]. Because delivery devices suitable for human use cannot be used in rodents, and because the instillation techniques used in rodents have no human counterpart, the preclinical and clinical versions of a nose-to-brain product are separated

by a translational gap in the administration step itself, independent of any biological difference [20].

This does not render rodent work valueless. It means that rodent work establishes mechanism and plausibility rather than magnitude, and that any preclinical report which projects a human benefit from a rodent targeting value is overreaching. Studies should state the anatomical basis of their species choice and should refrain from linear extrapolation.

5.5 Statistics, blinding and completeness

The remaining failures are generic to preclinical research but are aggravated here by the instability of the estimator.

Group sizes in this literature are typically small while the variance is high, a combination the standardised screening study identified as rendering conclusions questionable [9], and sample sizes are seldom justified by any prospective calculation. Randomisation to route or formulation, blinding of the analyst, and pre-specification of the primary outcome are rarely reported, and the omission matters more for a derived residual metric than for a directly measured endpoint, because the analytical latitude available after unblinding is correspondingly greater. Exclusions, which the contamination problem makes both legitimate and necessary, must carry their criteria and their number, since undisclosed exclusion of contaminated samples in one direction only would bias the result systematically.

5.6 The absent absolute

Finally, and underlying much of the above, the literature reports performance almost exclusively in relative terms. A targeting efficiency above one hundred percent states that the nasal route partitions more favourably than the intravenous route. It does not state what fraction of the administered dose reached the brain, and so permits no judgement about whether the quantity delivered could plausibly be therapeutic. A field that never reports the absolute fraction cannot detect the situation in which every formulation in a comparison performs poorly, and that is precisely the situation the analogous survey in tumour-directed nanomedicine eventually exposed [10]. Reporting the recovered fraction of administered dose alongside the relative metrics costs nothing and would restore a dimension of judgement the field has lost.

6. Why the existing frameworks are not sufficient

Three reporting frameworks bear on this literature, and each addresses a real deficiency without addressing the route-specific one.

ARRIVE 2.0 is the general standard for reporting in vivo experiments, organised as an Essential 10 covering study design, sample size, inclusion and exclusion criteria, randomisation, blinding, outcome measures, statistical methods, experimental animals, experimental procedures and results, with a recommended set covering context, ethics, husbandry, interpretation, generalisability, registration, data access and interests [22]. It was revised because the original

guidelines, despite broad journal endorsement, were not being followed. Evidence on adherence is sobering and directly relevant to any proposal of the present kind. A comparison of papers in endorsing and non-endorsing journals found no meaningful difference in adherence, with bias-related items poorly reported in both [23], and a cross-sectional analysis of 943 interventional animal studies found that essentially no study reported the full item set [24]. That analysis also found that journals which mandated the guidelines achieved markedly higher reporting quality than those which merely endorsed them, which identifies the mechanism that makes a standard effective. Endorsement is decorative. Mandate is not.

SYRCLE's risk of bias tool adapts the Cochrane framework to animal intervention studies, with ten entries spanning sequence generation, baseline characteristics, allocation concealment, random housing, blinding of caregivers and investigators, random outcome assessment, blinding of outcome assessors, incomplete outcome data, selective outcome reporting and other sources of bias [25]. It is the appropriate instrument for appraising internal validity in this field and is seldom applied to it. Its limitation for present purposes is that it is deliberately generic: none of its ten entries interrogates instillation volume, dissection protocol or comparator formulation, because no generic instrument could.

MIRIBEL addresses the bio-nano literature specifically, requiring minimum information across material characterisation, biological characterisation and experimental protocol, on the reasoning that variable reporting blocks reproduction, cross-study comparison, meta-analysis and computational modelling of the bio-nano interface [26]. Since a substantial share of nose-to-brain research is nanoparticulate, MIRIBEL compliance would eliminate an important source of the unrecorded variance documented in Section 4.4. It does not address the route, the comparator or the anatomy.

The gap is therefore specific and identifiable. Applied together, the three frameworks would secure the internal validity of the animal experiment and the characterisation of the material. None would compel a study to report the volume instilled, the depth of instillation, the animal's posture, the composition of the comparator arm, the dissection and perfusion protocol, or the regional resolution of tissue sampling. Those are the variables that determine whether a direct-transport claim is supportable. Broader consensus work on nasal delivery has converged on adjacent priorities, identifying critical quality attributes by product type and the validation of experimental and simulation models as research needs [27], and recent syntheses have proposed evaluation frameworks spanning targeting, pharmacokinetics, safety, device compatibility and anatomical translation [19]. What has not been produced is an item-level reporting standard for the preclinical study itself.

7. A reporting standard for preclinical nose-to-brain studies

7.1 Design of the standard

The standard proposed in Table 2 is deliberately modest in ambition and specific in content. It does not prescribe how experiments should be designed, because legitimate designs vary with the question, and a standard that dictated methodology would be resisted and ignored. It prescribes what must be disclosed, on the principle that a fully disclosed imperfect study is interpretable and correctable while an undisclosed perfect one is neither.

The items are organised into six domains following the sequence of the experiment: administration, formulation and dose, the comparator, tissue handling and sampling, analysis and metrics, and interpretation and translation. Each item names a specific quantity or procedure, and each was selected because the appraisal literature reviewed in Sections 4 and 5 provides a reason to believe that its omission changes the interpretation of the result. The standard is intended to be used alongside ARRIVE 2.0 rather than in place of it, and alongside MIRIBEL where nanomaterials are involved, and it deliberately does not duplicate their generic items.

Two features are worth noting. First, several items require a negative disclosure: if an intranasal solution control was not included, the study must say so rather than remain silent, because a reader cannot otherwise distinguish absence from omission. Second, the analysis domain requires pre-specification of the primary metric, which is the single item most likely to be resisted and, on the evidence in Section 4.3, the single item most likely to reduce spurious findings.

7.2 Grading what a design can support

A reporting standard tells a reader what was done. It does not tell them what the study is entitled to conclude, and in a field where the same phrase is used for claims of very different evidential weight, an explicit grading scheme is the necessary complement. Table 3 proposes four tiers.

A Tier 0 study measures brain exposure after intranasal administration with no systemic comparator. It establishes that the compound reaches central tissue by some route and supports no statement about pathway. Much of the published literature sits here, while using the vocabulary of higher tiers.

A Tier 1 study adds a matched systemic comparator, permitting estimation of the residual and therefore a quantitative direct-transport claim, provided the comparator is dose-matched and formulation-matched and the sampling supports the exposure integrals.

A Tier 2 study adds anatomical resolution, separating olfactory bulb from other regions and reporting a regional pattern that is consistent or inconsistent with the proposed pathway, together with vascular correction and a documented dissection protocol that make contamination and residual blood implausible as explanations.

A Tier 3 study adds a manipulation of the pathway itself, such as modulation of epithelial

permeability, pathway interruption, or a compartment-resolved imaging readout, so that the proposed mechanism is subjected to a test capable of failing. The perivascular work cited earlier exemplifies this structure [8].

The value of the scheme lies less in the labels than in the discipline of assigning one. An author who must declare a tier is an author who must confront the distance between the claim in the title and the design in the methods. A reviewer given the scheme has a concrete basis for requiring that the language of a manuscript be brought into line with its evidence, which is a more productive intervention than a general request for caution.

7.3 Implementation

Three practical conditions would determine whether such a standard changed practice, and the ARRIVE experience identifies all three.

Journals would have to mandate rather than endorse, since mandated frameworks substantially outperform endorsed ones in measured adherence [24]. A completed checklist submitted with the manuscript and published as supplementary material is the established mechanism, and specialist journals in drug delivery and pharmaceutics are the appropriate point of adoption, since they publish the bulk of this work.

Reviewers would need the grading scheme alongside the checklist, because a checklist verifies disclosure without constraining interpretation, and the mismatch between design and claim is what the scheme addresses.

Adoption would be accelerated if funders and reviewers treated the absolute recovered fraction and the intranasal solution control as expected components of a formulation study rather than optional additions. Both are inexpensive and both are diagnostic. Their routine inclusion would within a few years produce a literature in which the question of how much drug the nasal route actually delivers, and how much of any benefit is attributable to the carrier, could be answered from the published record rather than debated from it.

8. Discussion

The argument of this review can be compressed into a single observation. The nose-to-brain field has spent two decades improving the independent variable and neglecting the measurement instrument. Carriers have become more sophisticated, gels more responsive and ligands more selective, while the experimental apparatus used to judge them, namely the comparison against a systemic reference, the recovery of drug from tissue and the derived ratio, has not been standardised, validated or in many cases fully described. Progress in the independent variable cannot be detected through an uncalibrated instrument.

Three consequences follow that are worth stating directly.

The first concerns the existing literature. If the diagnosis offered here is correct, a substantial portion of published nose-to-brain results is not wrong so much as uninterpretable, in the specific sense that the information required to judge them

was not reported. This is a less damning conclusion than it may appear. Uninterpretable results are recoverable in principle, since many of the missing details exist in laboratory records, and a coordinated effort to collect them retrospectively, in the manner of the structured databases already assembled [7], would use resources better than generating further studies of the same design.

The second concerns the persistent disagreement between enthusiasts and sceptics. That disagreement has been conducted largely as a dispute about whether direct transport occurs. Framed that way it is not resolvable by the available evidence, and the two decades separating the original critique [4] from the recent clinical appraisal [5] demonstrate as much. Framed as a dispute about what a given design can support, it becomes tractable, and the tiered scheme in Table 3 shows why: the sceptical position is essentially the observation that most studies are Tier 0 or Tier 1 while claiming Tier 2 or Tier 3 conclusions. That is a criticism of inference rather than of biology, and one that better-specified studies can answer.

The third concerns translation. The anatomical and device arguments in Section 5.4 imply that the human olfactory route is a narrower target than the rodent equivalent, that conventional devices engage it poorly, and that the preclinical and clinical administration steps have no common ground [20,21]. A field that took this seriously would place less weight on rodent targeting magnitudes and more on mechanistic studies establishing which pathway operates under what conditions, and on deposition studies conducted in human-relevant models. It would also treat the trigeminal route with the seriousness its accessible surface area warrants, rather than as a secondary pathway mentioned in passing.

Certain limitations should be acknowledged. This is a narrative appraisal, and its selection of sources, while systematically searched, was not conducted under a registered protocol with duplicate screening, so selection effects in the methodological literature examined cannot be excluded. The reporting standard in Table 2 rests on documented failure modes rather than on a formal consensus process, and a Delphi exercise involving investigators, device scientists, analytical chemists and journal editors would be the appropriate next step before any claim to consensus status. The tier scheme is likewise a proposal, and its boundaries would benefit from testing against a sample of published studies to confirm that independent assessors apply them consistently.

9. Future directions

Four specific programmes of work would do more for this field than further formulation iteration.

A formal consensus exercise should convert the reporting items in Table 2 into a validated checklist. The model is available in the development history of both ARRIVE 2.0 and MIRIBEL [22,26], and the constituency for it already exists in the consensus activity around nasal delivery more broadly [27].

A retrospective reporting audit should then be conducted against those items, on a defined sample of preclinical nose-to-brain publications, to quantify the prevalence of each omission. This would establish a baseline against which later improvement could be measured and would identify which items are most urgently missing. Such an audit is an achievable systematic review, reportable under PRISMA 2020 [29] and appraisable with SYRCLE [25], and it requires no laboratory work.

An interlaboratory comparison should be undertaken in which several laboratories perform a nominally identical intranasal study on a common reference compound under a shared protocol. The pooled analyses in Section 4.4 infer the existence of large unrecorded methodological variance; an interlaboratory study would measure it directly and attribute it to specific steps. Nothing would do more to settle how much of the field's scatter is biological.

Finally, deposition-resolved studies in human-relevant models, coupling device performance to regional deposition and then to a measured transport readout, would address the translational gap where it actually opens, which is the administration step rather than the biology [20,21].

10. Conclusion

Direct nose-to-brain transport is an anatomically plausible phenomenon that has been investigated for two decades without arriving at a settled quantitative account of its contribution. The evidence assembled here indicates that the obstacle is the design and reporting of the studies rather than the phenomenon itself. Direct transport is a claim about route, its estimation depends on a residual computed against a systemic reference, and the experimental steps that determine that residual are the steps the literature most often leaves undescribed. Generic reporting frameworks secure internal validity and material characterisation but do not reach the route-specific variables, and pooled analyses repeatedly detect variance that tracks no pharmaceutical property, which is what unrecorded methodology looks like when viewed in aggregate.

The remedy is neither costly nor novel in kind. A route-specific reporting standard, mandated rather than endorsed, paired with an explicit grading of what each design can support, would allow the accumulated literature to begin functioning as evidence. Until a study is required to state how much liquid was placed where, against what comparator, and with what separation between the dosing site and the tissue analysed, the field will continue to publish numbers that cannot be compared and to debate a question that better-specified experiments could answer.

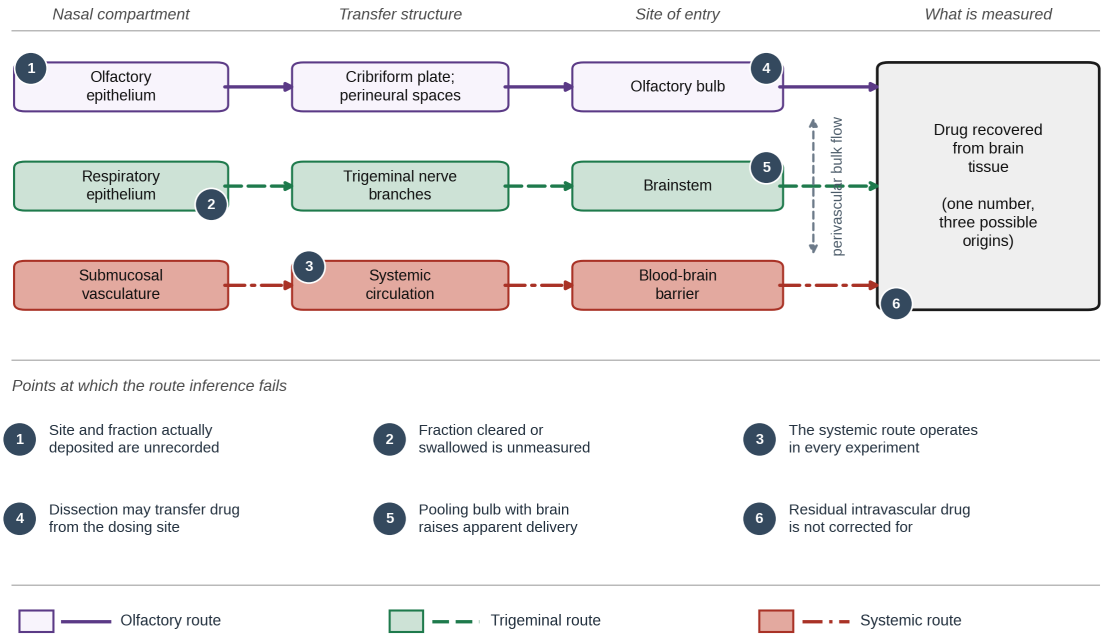

Figure 1. Three anatomically distinct routes from the nasal cavity converge on a single measured quantity, which is why recovery of drug from brain tissue cannot by itself identify the route taken.

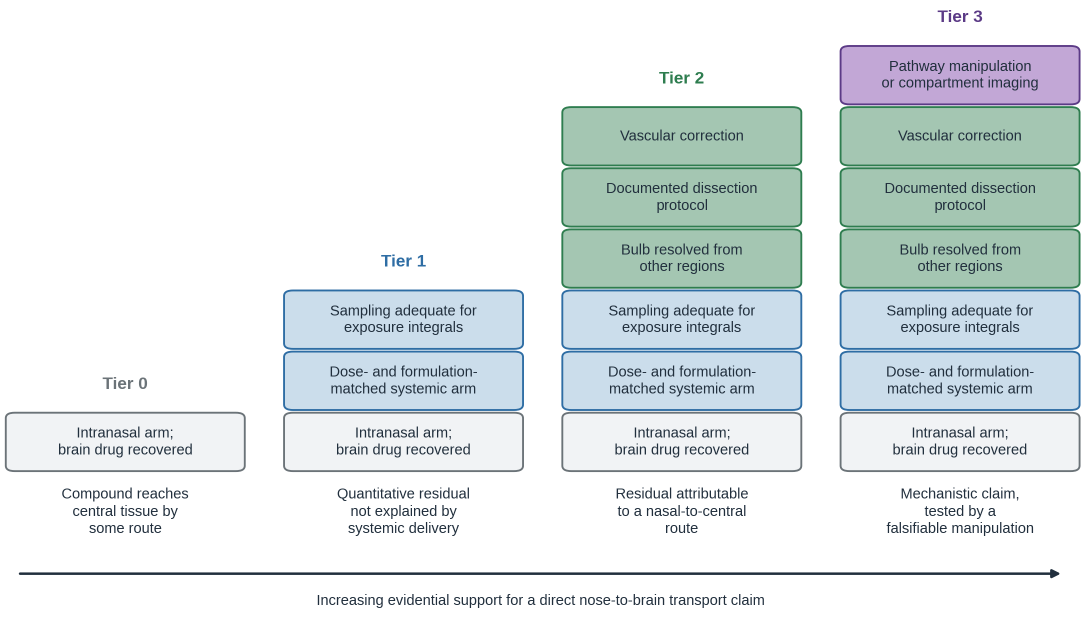

Figure 2. Evidence tiers for a direct nose-to-brain transport claim, showing that the tier a study occupies is determined by the design elements it contains rather than by the size of the effect it reports. Each column is one tier, and the blocks stacked within it are cumulative, so that every element required at a lower tier is also required at a higher one. Tier 0 comprises an intranasal arm alone. Tier 1 adds a dose-matched and formulation-matched systemic comparator together with a sampling schedule adequate to construct exposure integrals. Tier 2 adds anatomical resolution of the olfactory bulb from other regions, a documented dissection protocol and a correction for residual intravascular drug. Tier 3 adds an experimental manipulation of the proposed pathway or a compartment-resolved imaging readout. The text beneath each column states the

strongest claim that design can support; claims beyond that text are not licensed by the design, however large the measured difference. Block shading is separated in lightness so the tiers remain distinguishable in greyscale, and column height is itself the primary visual cue. The scheme is the proposal of this review and is set out item by item in Table 3.

Table 1. Derived metrics used to support direct nose-to-brain transport claims, the assumptions on which each depends, and the design failure that invalidates it. AUC denotes area under the concentration-time curve; subscripts IN and IV denote the intranasal and intravenous arms respectively.

Metric	Expression	What it asks	Core assumptions	Failure mode that invalidates it
Drug targeting efficiency (DTE%)	(AUC(brain,IN)/AUC(blood,IN)) ÷ (AUC(brain,IV)/AUC(blood,IV)) × 100	For a given blood exposure, does the nasal route yield more brain drug?	Systemic disposition independent of route; complete AUC in all four terms; comparable dose range	Comparator given as solution while nasal arm is a formulation; truncated sampling schedules; non-linear disposition between arms
Direct transport percentage (DTP%)	(B(IN) − B(x))/B(IN) × 100, where B(x) = AUC(brain,IV) × (AUC(blood,IN)/AUC(blood,IV))	What fraction of brain exposure is unexplained by systemic delivery?	All DTE assumptions, plus linearity of the brain-to-plasma relationship across the exposure range spanned	Small residual computed as the difference of two larger noisy terms; instability rises sharply at small group sizes
Drug targeting index (DTI)	Ratio of brain-to-blood exposure ratios, variably defined across reports	Same question as DTE, without a fixed convention	A stated and consistent definition	Definitional drift between papers; not comparable across the literature without checking each formulation
Tissue-to-blood ratio at sacrifice (C(br)/C(bl))	Single-timepoint concentration ratio	Snapshot of partitioning at one moment	Sampling time representative of the whole profile	Diverges from exposure-integral metrics when brain and plasma profiles differ in shape, so the same data can yield opposite conclusions [9]
Absolute recovered fraction (% of administered dose in brain)	Mass in brain ÷ mass administered × 100	How much drug actually arrived?	Accurate knowledge of the delivered rather than nominal dose	Rarely reported in this field, so therapeutic plausibility cannot be judged from the published record [10]

Table 2. Proposed minimum reporting items for preclinical studies asserting direct nose-to-brain transport. The standard is intended for use alongside ARRIVE 2.0 [22] for generic in vivo reporting and MIRIBEL [26] where nanomaterials are involved, and it deliberately omits items those frameworks already cover. Items marked with an asterisk require an explicit negative statement where the element was not included, so that a reader can distinguish absence from omission.

Domain	Item	What must be reported
A. Administration	A1	Instilled volume per nostril and in total, with the species and approximate nasal cavity capacity against which it should be read
	A2	Delivery method (pipette, cannula, catheter, spray, device) and, for instrumented delivery, the insertion depth

	A3	Animal posture during and after dosing, including head angle and the time held in position
	A4	Anaesthetic agent, dose and depth, or explicit statement that dosing was performed in conscious animals
	A5	Whether dosing was unilateral or bilateral, and the interval between nostrils if sequential
	A6	Number of operators, and any operator-level standardisation or blinding of the dosing step
B. Formulation and dose	B1	Complete formulation composition, including all excipients, permeation enhancers and surfactants
	B2	Nominal administered dose and, where measurable, the delivered dose, with the basis of any difference
	B3	Viscosity, pH, tonicity and, for particulate systems, the MIRIBEL characterisation set
	B4*	Whether a residence-time or clearance readout was obtained for formulations claiming prolonged retention
C. Comparator	C1*	Presence and composition of an intravenous or other systemic comparator arm
	C2	Dose administered in the comparator arm and the basis on which it was matched to the nasal dose
	C3*	Whether the comparator received the same formulation as the nasal arm, or drug in simple solution
	C4*	Presence of an intranasal solution control permitting the formulation effect to be separated from the route effect
D. Tissue handling and sampling	D1	Full dissection protocol, including the order of steps and the measures taken to prevent transfer of material from the nasal cavity to central tissue
	D2*	Whether olfactory bulbs were analysed separately from the remainder of the brain
	D3	Anatomical regions sampled and their boundaries, including whether brainstem was resolved separately
	D4*	Perfusion or flushing procedure, and any correction applied for residual intravascular drug
	D5	Sampling times, number of animals per time point, and the basis of the schedule
	D6	Analytical method, lower limit of quantification, and validation in the relevant matrix
E. Analysis and metrics	E1	The primary metric, stated as pre-specified or post hoc
	E2	Method of AUC estimation, the extrapolated fraction, and the treatment of values below quantification limits
	E3	All metrics computed, including any that were calculated and not selected as primary
	E4*	Absolute fraction of the administered dose recovered in brain, or a statement that it could not be determined
	E5	Sample size justification, randomisation, blinding of the analyst, and all exclusions with their criteria and number
F. Interpretation and translation	F1	The evidence tier claimed (Table 3), with the design features that support it
	F2	Explicit statement of which pathway the regional distribution pattern is consistent with, or that it does not discriminate
	F3	Anatomical basis of the species choice, and an explicit statement that targeting magnitudes are not linearly extrapolable to humans
	F4*	Relationship, if any, between the administration method used and a device that could be used clinically

Table 3. Proposed tiers of evidential support for direct nose-to-brain transport claims. The tier a study occupies is set by its design, not by the size of the effect it reports, and authors are asked to declare a tier and confine their conclusions to it. Tier 3 corresponds to the design structure exemplified by compartment-resolved perivascular transport work [8].

Tier	Design elements present	Claim the design can support	Claim it cannot support
0	Intranasal arm only; brain drug recovered	The compound reaches central tissue after nasal administration by some route	Any statement about pathway, targeting advantage, or blood-brain barrier circumvention
1	Adds a dose-matched and formulation-matched systemic comparator with adequate sampling for exposure integrals	A quantitative estimate of the residual brain exposure unexplained by systemic delivery, with stated uncertainty	Attribution of that residual to a named pathway; exclusion of contamination or residual blood as explanations

2	Adds regional resolution (bulb separated from other regions, brainstem resolved), documented dissection protocol, and vascular correction	Attribution of the residual to a nasal-to-central route, with a regional pattern consistent or inconsistent with olfactory or trigeminal transfer	A mechanistic claim about the transfer process itself
3	Adds an experimental manipulation of the proposed pathway, such as modulation of epithelial permeability, pathway interruption, or compartment-resolved imaging	A mechanistic claim about the operating pathway, tested by a manipulation capable of falsifying it	Quantitative extrapolation of magnitude to the human, which remains limited by the anatomical and device arguments of Section 5.4

Abbreviations

AUC, area under the concentration-time curve; ARRIVE, Animal Research: Reporting of In Vivo Experiments; CNS, central nervous system; CSF, cerebrospinal fluid; DTE, drug targeting efficiency; DTI, drug targeting index; DTP, direct transport percentage; MIRIBEL, Minimum Information Reporting in Bio-Nano Experimental Literature; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; SYRCLE, Systematic Review Centre for Laboratory Animal Experimentation.

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