The Use of Non-Technical Summaries to Sustain The Replacement, Reduction and Refinement in Respiratory Diseases Research

Introduction

A fundamental aspect driving high quality scientific research is the *accurate* and *transparent* reporting of data. Respiratory Diseases (RD) constitute a significant burden for nowadays society: in 2018, the World Health Organisation (WHO) ranked RD as the third leading cause of morbidity in the World (1) and most evidently the COVID-19 pandemic, which has brought to the forefront the deleterious effects that RD (e.g. SARS-COV-2) can have on global healthcare, economics and society (2). One other aspect that the COVID-19 pandemic has highlighted is the urgent need of accurate, human-directed RD therapies as well as the importance of sharing *accurate* and *transparent* information (3).

The 3Rs (i.e. Replacement, Reduction, Refinement) comprise of a set of guidelines committed to improving the quality of scientific research by sustaining the development of valid, reliable and *accurate* scientific procedures as well as the *transparent* and accessible reporting of data (4). It is well known that the public's opinion on matters such as the use of animal models in biomedicine enormously impacts on the funding prospects and regulatory processes (5). Particularly in the past, the scientific community has been reticent in reporting details regarding animal scientific procedures, often producing an overall lack of trust on the public's behalf (6).

To tackle such issues, in September 2010, the European Union (EU) introduced the 63/2010/EU Directive with the intention of implementing "more stringent and *transparent* measures in the area of animal experimentation" (7). All member states (United Kingdom included) adhered to such legislation in order to enhance and standardize a set of regulations aimed at producing more human - and less animal - based experimental procedures. Among the many strategies adopted by the EU member states, one was the publication of Non-Technical Summaries (NTS): official documents that researchers must present to the competent authorities (i.e. Animals in Science Regulation Unit; ASRU UK) in order to receive a licence to undertake science projects involving the use of animals.

A similar but less *transparent* strategy implemented by the 63/2010/EU Directive was the introduction of Retrospective Assessments (RA) – documents required along with NTS submissions that propose the use of non-human primates, dogs, cats, equines and endangered animals in procedures exceeding severe severity levels and for educational training purposes (8). Although, RA were not conceived for immediate publication on the Home Office Website like NTS, their introduction represented a solid attempt in strengthening animal research regulatory measures.

Research Aims, Objectives and Hypotheses

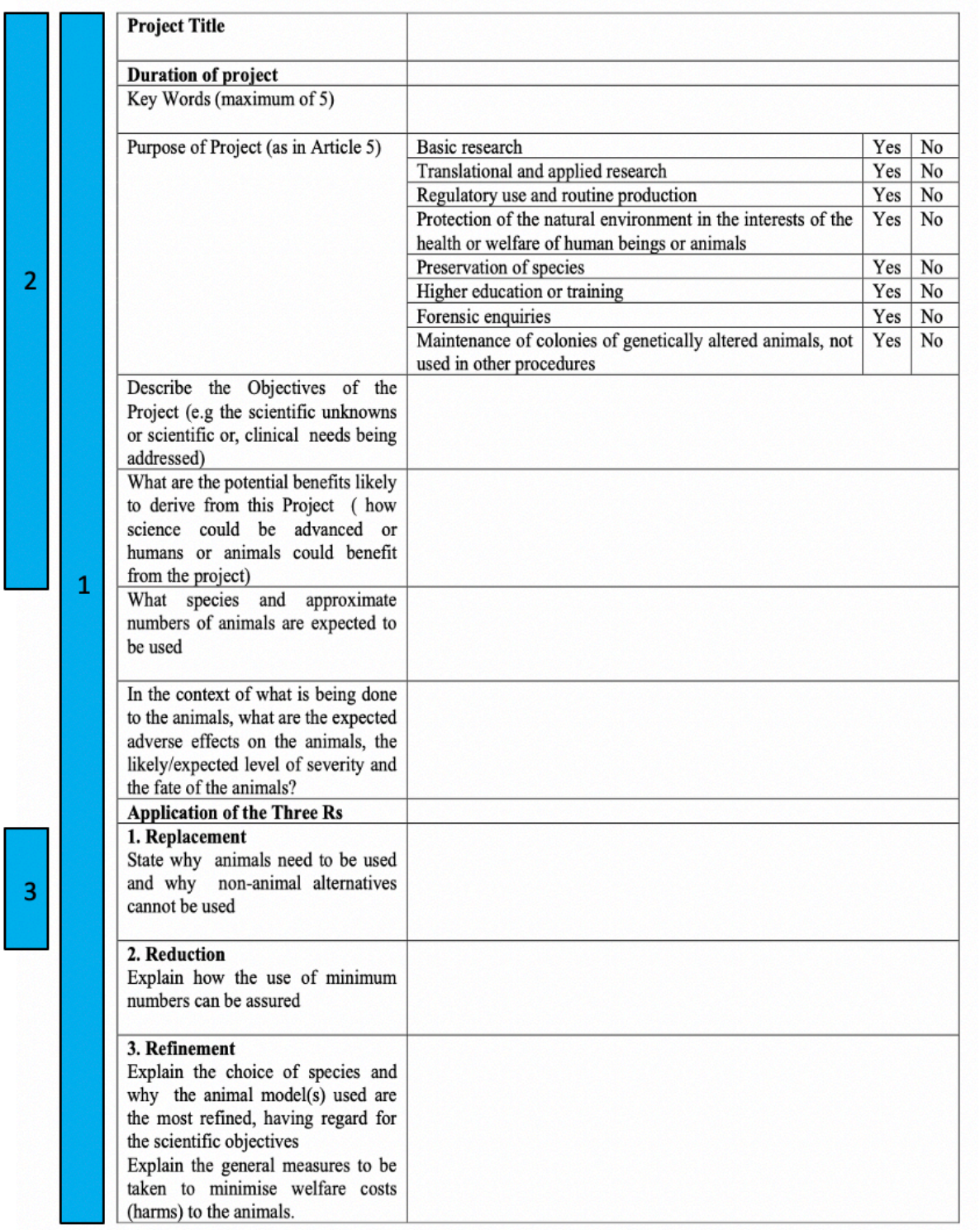
The intention of this Studentship award was to analyse NTS as the only, freely accessible source of scientific material available to the general public concerning the implementation of the 3Rs in laboratories. The aim of this project was therefore to critically analyse NTS and evaluate whether the 3Rs reporting system in the UK has been undertaken *accurately* and *transparently*.

The main aim of this project was to create a systematic literature review by critically analysing all NTS related to RD research granted by ASRU since 2013, when the 2010/63 EU directive was actively adopted. This project also intended to elucidate significant trends in animal use within RD research from 2013 to 2019 and to identify areas and projects of respiratory research that require further advancement for *in vitro* alternatives. Additionally, this investigation sought to discern factors that may have influenced the choice of animal species utilised by researchers despite the availability of a plethora of validated alternative models for lung research (9). Finally, an ultimate goal was to ascertain whether alternative research methods were duly explored for the Replacement (i.e. 1R) of laboratory animal testing by the researchers who wrote the NTS.

The objective of this literature review was to accept or reject the hypotheses and to identify potential areas within the NTS literature that requires motivation to seek alternative methods that replace (i.e. 1R) animal testing. Therefore, based on the fact that all NTS applications granted by the ASRU UK utilise the same pro-forma design (**Figure 1**), the hypotheses for this literature review project were three-fold (**Table 1**).

Table 1. An overview of the research project's hypotheses.

Hypothesis number	Hypothesis statement
1	The NTS were *accurate*, *transparent* and followed the guidelines given by the 2010/63/EU Directive
2	The NTS proposed studies were relevant to the advancement of RD
3	The use of *in vivo* models was justified by the lack of alternative *in vitro* approaches



Project Title			
Duration of project			
Key Words (maximum of 5)			
Purpose of Project (as in Article 5)	Basic research	Yes	No
	Translational and applied research	Yes	No
	Regulatory use and routine production	Yes	No
	Protection of the natural environment in the interests of the health or welfare of human beings or animals	Yes	No
	Preservation of species	Yes	No
	Higher education or training	Yes	No
	Forensic enquiries	Yes	No
	Maintenance of colonies of genetically altered animals, not used in other procedures	Yes	No
Describe the Objectives of the Project (e.g the scientific unknowns or scientific or, clinical needs being addressed)			
What are the potential benefits likely to derive from this Project (how science could be advanced or humans or animals could benefit from the project)			
What species and approximate numbers of animals are expected to be used			
In the context of what is being done to the animals, what are the expected adverse effects on the animals, the likely/expected level of severity and the fate of the animals?			
Application of the Three Rs			
1. Replacement State why animals need to be used and why non-animal alternatives cannot be used			
2. Reduction Explain how the use of minimum numbers can be assured			
3. Refinement Explain the choice of species and why the animal model(s) used are the most refined, having regard for the scientific objectives Explain the general measures to be taken to minimise welfare costs (harms) to the animals.			

Figure 1. Schematic diagram denoting the NTS pro-forma; adapted from (10). This consistent organisation allowed for keywords searches; analysis of projects' purposes & objectives; extrapolatiton of significant indices of NTS *transparency* and *accuracy* such as animal numbers, animal species, harm-pain severity levels, period of animal use. The section named **Application of the Three Rs** provided important data regading the 3Rs employment in NTS experimentations. This denotes that specific standardised sections were used in order to test each NTS project's hypothesis.

Materials & Methods

NTS Source & Selection

The very first obstacle encountered was the selection of specific NTS related to RD. In order to do so, the *Medical Subject Headings 2021* (MeSH) browser (11) was used to create a list of "Respiratory Diseases Research" and "Animals Testing" related keywords. However, based on the notion that NTS were written for a lay audience, another search was made via the browser *Power Thesaurus* (12) designed for the search of less jargonised keywords and synonyms related to "Respiratory Disease", "Lungs", "Animal Testing". Subsequently, NTS were accessed via the Home Office Website (13) and selected based on their *title* and *keywords* similarity to the lists of words that were created with MeSH and Power Thesaurus.

Changes To The Plan Firstly Presented To FRAME

Initially, the project's plan was to critically analyse the most recently published NTS which were granted in 2018. However, only 20 NTS were found to be related to RD research in the 2018 NTS Volume. Therefore, we considered doing a temporal study based on all NTS granted between 2013 and 2018 related to the field of RD. This decision was made with the intention to create a more comprehensive analysis to provide a more inclusive and current perspective of the NTS reporting system quality. The project was planned and started in June 2020, however three months later, in September 2020, the NTS granted in 2019 by ASRU were released on the UK Home Office official website. Therefore, the total number of NTS comprised in this study was increased to 174.

Data Analysis

Once all the NTS were selected, data relevant to our case study was extracted (see **Figure 1**) and reported into five matrix tables (i.e. [1] abstract analysis; [2] project's purpose; [3] replacement; [4] reduction; [5] refinement) designed after an extensive literature search of the 3Rs and current validated alternative strategies (14).

NTS Marking Criteria

A summary of the NTS marking critieria **(Table 2)** provides an outline of the criteria (i.e. Grammar & Syntax, Lay Terminology and Statements Specificity) based on which NTS were given a particular Score for '*Accuracy* and *Transparency*'. These NTS criteria were devised using the official British NTS reporting guidelines (10), (15) and previous work done by Taylor and Weber (2018) on the analysis of NTS system's quality in Germany and the UK (16).

Table 2. Summary of the NTS marking criteria. NTS were awarded the score of 1 for each of the fulfilled criteria: Grammar & Syntax; Lay Terminology; Statements Specificity. The binary score system 0, 1 was designed to allow further statistycal analysis (17). *Summary system developed by M. Bonassera (2020)*.

Grammar & Syntax	**Lay Terminology**	**Statements Specificity**	**Score**
Grammar & syntax are always correct; **AND** the sentences are generally short (15-20 words).	The terminology and writing style used is generally accessible for lay readers; **AND** abbreviations are explained.	The statements are consistently valid and specific; **AND** the summary does not overstate the potential benefits; **AND** the aims & objectives are clearly outlined.	1 - Accurate
Grammar & syntax are generally incorrect; **OR** most sentences exceed 20 words length	The terminology and writing style is not accessible for lay readers; **OR** Abbreviations are not explained.	The statements are often vague and not supported by secondary literature; **OR** the summary overstates the potential benefits; **OR** the aims & objectives are not clearly outlined.	0 - Not Accurate

Conclusions

Across the past decade, various British competent organisations committed efforts to increase data *accuracy* and *transparency* in the field of reporting animal research – among the most famous are the Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) guidelines published in 2010 by the National Center for the Replacement, Reduction and Refinement of Animals in Research (NC3Rs). In July 2020, an updated verison of the ARRIVE guidelines (2.0 ARRIVE) was released (18). These guidelines complement the Planning Research and Experimental Procedures on Animals: Recommendations for Excellence (PREPARE) guidelines written and published in 2018 by Norecopa, the Norway's National Consensus Platform for the advancement of the 3Rs (19). Additionally, Understanding Animal Research UK along with the Basel Declaration Society intended to increase the level of detail in animal research by sighting standardised writing models (20). This collective effort is based on evidence showing that insufficient *transparency* regarding reporting data may contribute to the building up of a mis-informed public and biased opinions which may negatively impact (i.e. stagnate) current/future research development (21). Therefore, studies that assess the quality of NTS reporting in different fields of research could be a valuable source of information not only for the general public but also for competent authorities (**Table 3**) and the whole scientific community.

Table 3. List of the Europen 3Rs Centers cooperating with European Union Reference Laboratory for alternatives to animal testing - EURL ECVAM, (22).

Abbreviation	**Full name**	**EU State**
NC3Rs	The National Centre for the Replacement, Refinement and Reduction of Animals in Research	UK
FICAM	Finnish Centre for Alternative Methods	Finland
Norecopa	Norway's National Consensus Platform for the advancement of "the 3 Rs"	Norway
CAAT-Europe	Center for Alternatives to Animal Testing	Europe (affiliated with Johns Hopkins University, Baltimore, USA)
IZSLER	Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna	Italy
SWETOX	Swedish Toxicology Sciences Research Center	Sweden

ROCAM	Romanian Center for Alternative Test Methods	Romania
Bf3R	Federal Institute for Risk Assessment	Germany
RIVM	National Institute for Public Health and the Environment	The Netherlands
3Rs-Centre ULS	3Rs-Centre Utrecht Life Sciences	The Netherlands
FSVO	Federal Food Safety and Veterinary Office	Switzerland

Opportunities & Future Research

In the future, the aim is to produce a systematic literature review reporting data which assess the *accuracy* and *transparency* of all NTS published for animal use purposes in RD research. Although this study only focuses on the UK NTS reporting system, it would be interesting to see whether there are any differences or similarities between EU member States NTS reporting systems (16).

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