

Russell and Burch's 3Rs Then and Now: The Need for Clarity in Definition and Purpose

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Russell and Burch's *The Principles of Humane Experimental Technique* was first published in 1959. A Special Edition containing the original text was reissued in 1992, after its ideas had gained widespread interest in the scientific community. In the *Principles*, Russell and Burch proposed a new applied science that would improve the treatment of laboratory animals while advancing the quality of science in studies that use animals. They introduced and defined the terms *replacement*, *reduction*, and *refinement*, which subsequently have become known as 'alternatives' or 'alternative methods' for minimizing the potential for animal pain and distress in biomedical research. Here we describe and explain the original definitions of the 3Rs in the *Principles*, examine how current definitions differ among themselves and from Russell and Burch's definitions, and suggest relevant considerations for evaluating all definitions of the 3Rs.

Abbreviations: APHIS Policy 12, Animal Plant Health Inspection Service Animal Care Resource Guide, policy 12, *Consideration of Alternatives to Painful/Distressful Procedures*; AVMA Policy, American Veterinary Medical Association Policy on the Use of Animals in Research, Testing, and Education; CAAT, Center for Alternatives to Animal Testing; FRAME, Fund for the Replacement of Animals in Medical Experiments; ILAR, Institute of Laboratory Animal Research; ILAR Guidelines on Neuroscience Research, *Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research*; ILAR Report on Distress, *Recognition and Alleviation of Distress in Laboratory Animals*; *Principles, The Principles of Humane Experimental Technique*; UFAW, Universities Federation for Animal Welfare.

WMS Russell (1925–2006) and RL Burch (1926–1996) originated the concepts of *replacement*, *reduction*, and *refinement*, which they published in their 1959 book, *The Principles of Humane Experimental Technique*.¹⁶ Russell and Burch proposed a new applied science, the aim of which was to improve the treatment of research animals while advancing the quality of scientific and medical research and testing. The *Principles* was presented not as the final word of this science, but as a foundation for future developments. The 3Rs, as Russell and Burch first called them, were put forward not just to assist investigators in finding and using currently available techniques but also to encourage the development of as-yet unknown tools and methodologies, by expressing the fundamental goals of the new science. This is one reason why Russell and Burch declare in the *Principles* that "(a)ny applied science must have clearly defined aims, which in turn define priorities" (p 15).¹⁶ For Russell and Burch, it was thus of the utmost importance how the terms *replacement*, *reduction*, and *refinement* are *defined*.

Here we describe and explain Russell and Burch's definitions of the 3Rs. Some might think that such a discussion is unnecessary. Surely, everyone who has anything to do with the use of animals in research already knows how Russell and Burch defined the 3Rs. For years, these terms have appeared in virtually every context relating to the use of animals in research—in laws, regulations, and government policies; ethical pronouncements of professional research organizations; and books and journal articles. As is evidenced by this issue of *JAALAS*, there are important questions regarding how to promote *replacement*,

reduction, and *refinement* in various areas of animal research. But, one might suppose, what the 3Rs *themselves* are, how they are *defined*, certainly is not an issue; thanks to Russell and Burch, standard and universally accepted definitions of the 3Rs have long been in place.

In fact, as we illustrate, there is not unanimity regarding how *replacement*, *reduction*, and *refinement* are defined. Some definitions of the 3Rs that are now widely accepted (and claim the *Principles* as their source) differ among themselves and differ significantly from the definitions in the *Principles*. The situation is complicated further by the fact that Russell and Burch might have proposed an alternate definition of *refinement* and view of the overall aim of the new applied science.

Any satisfactory approach to the 3Rs must begin with clear definitions and with clearly stated and persuasive reasons for these definitions. Departing from the original definitions in the *Principles* would not be inappropriate—and indeed would be advisable—if changes represent improvements. Perhaps because they believe they are applying Russell and Burch's definitions of the 3Rs, most who accept definitions that depart from the originals do not supply supporting arguments for these departures. In any event, there is no better way to achieve the clarity of definition and purpose that Russell and Burch themselves demanded in the *Principles* than by starting with meticulous examination of the definitions of the 3Rs in their book. Because these definitions *are* accompanied by supporting explanations, they suggest relevant considerations for evaluating *all* definitions of the 3Rs.

We do not here summarize the *Principles* or describe in depth the techniques it discusses for effecting *replacement*, *reduction*, and *refinement*. Our focus is on the *definitions* of the 3Rs. Limitations of space allow consideration of only a representative sample of recent definitions. Readers are encouraged to

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compare Russell and Burch's definitions to other versions with which they may be familiar. Our primary purpose is to encourage serious consideration of how the 3Rs *should* be defined. We treat the definitions in the *Principles* as a baseline as it were and ask proponents of different definitions to clarify and provide supporting arguments for these differences. Michael Balls, a leading proponent of *Principles*, has observed that

although a large number of people say they are committed to supporting the 3Rs concept of reduction, refinement, and replacement as put forward by Russell and Burch, most of them ... have not read the book itself. The result is that ... the great benefits afforded by a careful consideration and dedicated application of the *Principles* have not been achieved (p 19–20).⁶

Because it is likely that many readers of this article and members of the research community have not read the *Principles* in its entirety, our discussion is necessarily extensive. There is however no substitute for the original, and we encourage readers to consult the *Principles* for further information and guidance about the nature and potential applications of the 3Rs.

The Concepts of Inhumanity and Humanity

Central to Russell and Burch's definitions of the 3Rs are 2 concepts first used by the *Principles*: *inhumanity* and its opposite, *humanity*. Humanity, as Russell and Burch understand it, is the ultimate goal of the 3Rs. However, the concept of inhumanity is in reality more fundamental, because the 3Rs seek to achieve humanity by diminishing and when possible eliminating inhumanity. Indeed, Russell and Burch discuss the nature of inhumanity at length and then characterize humanity as the absence of inhumanity. This treatment explains why the chapter of *Principles* that discusses the nature of both humanity and inhumanity is titled "The Concept of Inhumanity."

Inhumanity and humanity are descriptive and not normative terms. Russell and Burch use the terms *inhumanity* and *humanity* because they seek to further the *ethical* goal, stated by the Universities Federation for Animal Welfare (UFAW) "to promote humane behavior towards wild and domestic animals in Britain and abroad so as to reduce the sum total of pain and fear inflicted on animals by man" (p 14).¹⁶ However, their concepts of inhumanity and humanity do not themselves express value judgments but are strictly *descriptive* and *empirical*. The terms refer to objectively verifiable and measurable aspects of

the treatment of lower animals, specifically vertebrates....[T]he words will be used in a purely objective sense to characterize the kind of treatment actually applied to an animal—in terms of the effect on the latter. Our use of the terms, henceforward, therefore, MUST NOT BE TAKEN TO IMPLY ETHICAL CRITICISM OR EVEN PSYCHOLOGIC DESCRIPTION OF PERSONS PRACTICING ANY GIVEN PROCEDURE (p 14, capitalization in the original).¹⁶

The terms *inhumanity* (or *inhumane*) and *humanity* (or *humane*) are used in the *Principles* sometimes to refer to mental states experienced by experimental animals and sometimes to procedures or ways of treating animals that produce these mental states.

Inhumanity is equivalent to distress. Russell and Burch characterize inhumanity by describing a number of unpleasant mental states that can be experienced by research animals. Referring to the UFAW statement quoted earlier, they begin with pain and fear, stating that "we should like to replace these two specific conditions by the rather more general notion of distress" (p 15).¹⁶ These discussions of pain and fear are followed by consideration of several other unpleasant mental states that are also subsumed under the general category of *distress*. The first is *conflict*, which they characterize as a mental state in which an animal is beset by 2 or more opposing drives, which often results in fear. They provide the example of "so-called experimental neuroses," in which "the animal is normally driven into a situation where flight [from a painful stimulus] is either impossible or blocked by conflict with other drives. In such circumstances, fear must become an acutely unpleasant state which, by human analog may be termed *anxiety*" (p 22, italics in original).¹⁶ Russell and Burch subsequently add to the list of kinds of inhumanity another "category of distress states, to which definite rank may be assigned, namely states associated with frustration of a need. In this group we may include, e.g., hunger and bodily discomfort" (p 25).¹⁶ In the *Principles* when used to refer to mental states experienced by animals, the term *inhumanity* is synonymous with *distress*.

Russell and Burch prefer to talk about *distress* rather than simply *pain* or *fear*, for example, because the focus of the 3Rs is elimination or minimization of significantly *distressful* experiences in research animals. Considering mental states such as pain and fear is crucial, but not sufficient, because it is possible that an animal (or human) can experience pain, fear, or another mental state typically associated with distress but is not bothered by that mental state. For example, they note that although fear is usually unpleasant, "it need not be distressing, provided it has the opportunity for expression in effective action. In these conditions, it actually heightens and broadens awareness of environmental factors" (p 21).¹⁶

Another reason Russell and Burch subsume all mental states that constitute inhumanity under the general rubric of *distress* is that they do not want to restrict inhumanity to the specific kinds of unpleasant experiences (pain, fear, conflict, hunger, and bodily discomfort) discussed in the *Principles*. All forms of significantly distressful feelings qualify as distress—and for diminution and when possible elimination by application of the 3Rs. Russell and Burch explain distress that constitutes inhumanity as follows:

We may, then define distress of a certain degree (of whatever origin) as a central nervous state of a certain rank on a scale, in the direction of the mass autonomic response *which if protracted, would lead to the physiologic stress syndrome*. Inhumane procedures are those which drive the animal's mood down in rank toward this point. Removing inhumanity must ultimately mean driving the animal as near the *other* end of the scale as we can. "More humane" then simply means "less inhumane" in the above sense. ... We need only add that inhumanity can take two forms—acute and chronic—with no doubt every possible gradation between the two (24, italics in original).¹⁶

The first sentence of this passage is unclear. It may mean that a given unpleasant mental state would not qualify as distress unless this state, if protracted, would lead to the physiologic stress syndrome. This interpretation is supported by the state-

ment that *distress* of a certain degree is *defined* in terms of its leading, if protracted, to the physiologic stress syndrome. Alternatively, Russell and Burch may simply be saying that all mental states that constitute distress can be arranged on a scale ranging from distress that would, if protracted, lead to the physiologic stress syndrome to distress that is far less unpleasant and ultimately to no distress at all, and that the best way of conceiving of different levels of distress is to view them as occupying different positions on this scale. This interpretation seems supported by the subsequent statement, at the beginning of a chapter section titled “The Criteria for and Measurement of Distress,” that “(i)n principle, then, we can determine the presence of distress, and define a measurable amount of it in terms of rank on the scale. When we consider acute changes, any treatment which induces a mood lower in rank than the preexisting one may be thought of as imposing a measurable amount of distress” (p 24).¹⁶

In any event, Russell and Burch clearly believe that it is usually possible to quantify distress with sufficient precision to be able to place the levels of distress that animals experience on a scale with extreme distress at one end and decreasing distress as one moves toward the other end of the scale. The aim of the science of humane experimental technique is to move the level of distress, or inhumanity, experienced by research animals lower and lower on the scale. Each decrease in distress or inhumanity is necessarily an equivalent increase in humanity. As distress or inhumanity is decreased more and more, an experimental procedure or a kind of animal research becomes increasingly humane. Russell and Burch appear to state that when distress or inhumanity are completely absent, animals are not being treated inhumanely, but humanely.

Direct and contingent inhumanity. Central to Russell and Burch’s concept of inhumanity, and to the 3Rs, is their distinction between *direct* and *contingent* inhumanity. They define the former as “the infliction of distress as an unavoidable consequence of the procedure employed, as such, even if it is conducted with perfect efficiency and completely freed of operations irrelevant to the object in view” (p 54).¹⁶ Examples of direct inhumanity would be a procedure that necessarily inflicts unrelieved pain in an experiment designed to test the effectiveness of a pain-killing drug and a procedure regarding which there is as yet no available means of relieving some associated distress. In contrast, *contingent inhumanity* is defined as “the infliction of distress as an incidental and inadvertent by-product of the use of the procedure, which is not necessary for its success.” (p 54) Examples of contingent inhumanity include poor husbandry and handling techniques that cause unnecessary distress and ineffective methods of euthanasia. The *Principles* provides extensive descriptions of various techniques for reducing or eliminating direct and contingent inhumanity.

Individual and total inhumanity. In presenting the 3Rs as ways of reducing or eliminating inhumanity, the *Principles* aims at reducing and eliminating distress felt by individual animals. However, just as important in the program of the 3Rs is diminishing as much as possible the *sum total* of inhumanity or distress experienced by *all* animals used in a given experiment, in a kind of animal research or testing, and in animal research and testing generally. As noted earlier, a statement fundamental to the new science of humane experimental techniques is the declaration of UFAW of its aim “to promote humane behavior towards wild and domestic animals in Britain and abroad so as to reduce the sum total of pain and fear inflicted on animals by man.” Russell and Burch call this statement an “admirable guide” for their new science (p 15).¹⁶ They comment that

the quantitative aspect of this applied science is plainly set out in the words ‘sum total’. Ideally, if we could measure pain and fear on a perfected graded scale and with complete accuracy in practice, we should doubtless conclude that this sum total is made up by the sum of a number of products—each consisting of a certain definite amount of pain or fear multiplied by the number of animals exposed to it. In practice, without anything more than the barest approach to a realization of this idea, we may reasonably allot priorities in terms of either extreme unpleasantness or very large numbers of animals or a combined estimate of the two (15).¹⁶

What Russell and Burch mean in the final sentence is not entirely clear, but they may be acknowledging the possibility of conflict between the aim of minimizing inhumanity experienced by individual animals and minimizing the sum total of inhumanity experienced by all animals in an experiment. For example, it may sometimes be possible to inflict roughly the same total amount of distress either by using fewer animals, each of which experiences more distress, or more animals, each of which experiences less distress. In such circumstances, assuming the scientific results of the alternative approaches would be comparable, a choice of priorities must be made. Russell and Burch do not indicate what their choice would be in such circumstances, but such choices seem to be ethical (based on what one thinks it is right to do to the animals) and not purely scientific.

The Definitions and Fundamental Aim of the 3Rs

Diminution and removal of distress. After discussing inhumanity and distress, Russell and Burch first mention and briefly define the 3Rs in the fourth chapter of the *Principles*, titled “The Sources, Incidence, and Removal of Inhumanity,” in a section of this chapter titled “The Removal of Inhumanity: The 3Rs.” The importance of these titles cannot be overestimated. They—and the subsequent chapters of the *Principles* that discuss the 3Rs and their role in removing inhumanity—provide incontrovertible evidence of what the definitions of the 3Rs themselves make absolutely clear: the purpose of *all* 3Rs is the diminution and—when possible—removal of inhumanity or distress. Russell and Burch introduce the 3Rs as follows:

We turn now to consideration of the ways in which inhumanity can be and is being diminished or removed. These ways can be discussed under the 3 broad headings of Replacement, Reduction, and Refinement.... (T)he 3 modes now considered have conveniently been referred to as the 3Rs of humane technique.

Replacement means the substitution for conscious living higher animals of insentient material. Reduction means reduction in the numbers of animals used to obtain information of a given amount and precision. Refinement means any decrease in the incidence or severity of inhumane procedures applied to those animals which still have to be used (p 64).¹⁶

We provide detailed explanations of these definitions later. However, because of the frequency of mischaracterizations of the definitions, it must again be emphasized that *all* 3Rs are

presented as “the ways in which inhumanity can be and is being diminished or removed.” Replacement minimizes research animal distress by substituting animals that can experience distress with insentient material that is incapable of feeling anything and therefore cannot experience distress. Reduction minimizes research animal distress by decreasing the number of animals that can experience distress. Refinement is, by definition, diminution or elimination of distress. This definition of refinement is not intended to suggest that replacement and reduction have a different aim. Refinement is presented as a distinct method of removing inhumanity because it focuses on the actual conduct of research, and on how sentient research animals are treated.

Importance of research aims and scientific and medical progress. Although the fundamental aim of the 3Rs is diminishing or removing distress, for Russell and Burch this aim—and use of the 3Rs—cannot be allowed to compromise the goals of conducting sound science and achieving scientific and medical progress. Russell and Burch declare that the “central problem” faced by their proposed science of humane experimental technique “is that of determining what is not humane, and how humanity can be promoted without prejudice to scientific and medical aims” (p 14).¹⁶ The goal of minimizing inhumanity is not to be balanced *against* the aims of a scientifically sound experiment or kind of research, so that these latter aims must sometimes give way to or be modified in the interests of inflicting less distress on animals.

The 3Rs and efficiency. Importantly, however, Russell and Burch also maintain that there is a convergence of high-quality research and the use of the 3Rs to minimize inhumanity. They proclaim that “it is widely recognized that the humanest possible treatment of experimental animals, far from being an obstacle, is actually a prerequisite for successful animal experiments. Since the Second World War, in particular, this principle has been increasingly accepted; and the intimate relationship between humanity and efficiency in experimentation will recur constantly as a major theme in the present book” (p 3–4).¹⁶ Many discussions in the *Principles* seek to demonstrate that experiments achieve better scientific results when animals experience no distress or the least possible distress consistent with experimental aims. By “efficiency,” Russell and Burch also mean generating maximum scientific or medical results from expenditures of monetary and animal resources, facilities, and personnel. They maintain that such resources are often wasted, or do not achieve the best results, when animals suffer distress unnecessarily.

The 3Rs and Alternatives

The term *alternatives* is commonly used—and attributed to the *Principles*—as referring to the 3Rs. For example, Policy 12 of the US Department of Agriculture Animal Plant and Health Inspection Service (APHIS Policy 12), which explains the provision of the Regulations of the Animal Welfare Act requiring investigators to consider the use of alternatives to painful or distressful procedures,³ states that “(a)lternatives or alternative methods, as first described by Russell and Burch in 1959, are generally regarded as those that incorporate some aspect of replacement, reduction, or refinement of animal use.”¹⁷

In fact, the terms *alternatives* and *alternative methods* never occur in the *Principles*. Balls recounts that Burch was the first person to use the term *alternatives*, before he started working with Russell on the *Principles*. However, in preparing the final manuscript Russell rejected Burch’s suggestion that the term be used to describe the general theme of the book (p 258).⁵ Russell believed that physiologist David Smyth first used *alternatives*

to apply specifically to the 3Rs (p 279),¹⁴ in his 1978 book, *Alternatives to Animal Experimentation*.¹⁸ Use of the term was popularized by the UK Fund for the Replacement of Animals in Experiments (FRAME), which is credited with rediscovering and promoting the *Principles* 2 decades after its publication.⁴ Because of the support and promotion of their work by FRAME, the Johns Hopkins Center for Alternatives to Animal Testing (CAAT), and others, Russell and Burch understood that *alternatives* came to be widely used to refer to the 3Rs.¹⁷ However, in a 1995 lecture Russell agreed with Alan Goldberg, then Director of CAAT, that the word is “unfortunate” because it suggests only 1 R, replacement. “I have been pleased to note,” Russell continued, “that in the last couple of years this confusing term appears to be on the way out.” (p279).¹⁴

That *alternatives* came to be associated with the 3Rs by *others*—and well *after* publication of the *Principles*—is of more than just historical interest. As we discuss later, almost all contemporary discussions of the 3Rs erroneously attribute to the *Principles* the definition of *replacement* as the use of nonanimal materials. This interpretation may have derived not from a careful reading (or any reading) of the *Principles*, but from the fact that both FRAME and Smyth not only called all 3Rs *alternatives* but also defined *replacement* to mean the total elimination of animals in research. Smyth, for example, after declaring that Russell and Burch’s 3Rs “still remain the best approach to alternatives,” characterized *replacement* as “any procedures which do away with the use of animals altogether” (p 14).¹⁸ The term *alternatives* itself suggests the use of research materials *other than* animals.

Wellbeing—the Aim of Refinement and the 3Rs?

A brief discussion in the *Principles* might assert that wellbeing—and not the diminution and removal of distress—is the primary aim of the new science of humane experimental technique. In the context of the book as a whole, this passage (which consists of 2 paragraphs) is an anomaly. The views it might express are not taken up elsewhere. The discussion is also unclear and confusing. However, the passage requires thorough attention in light of the fact that some contemporary definitions of refinement include promotion of animal *wellbeing*. Moreover, decades after publication of the *Principles*, Russell and Burch may have repeated some of the statements made in the passage.

What is wellbeing? Before we discuss this passage in the *Principles*, and recent definitions of refinement that include promotion of wellbeing, it is crucial to note that the term *wellbeing* does not have a single, self-evident, and universally accepted meaning. The term has been used in the animal research literature to mean simply the absence of distress, but it also can be and has been used to refer to a number of different positive mental states—ranging from very mild and brief feelings of comfort; to feelings of great comfort; to satisfaction resulting from eating, drinking, and the fulfillment of certain basic physiologic needs; and to mild pleasures, intense pleasures, feelings of happiness, and happy lives.²⁰ Any characterization of the aim of the 3Rs that includes promotion of wellbeing but does not define the term *wellbeing* makes it impossible to know what researchers are being asked to promote, whether certain techniques promote such wellbeing, and whether promotion of such wellbeing might advance or compromise the aims of research projects.

The problematic discussion of wellbeing in the *Principles*. The term *wellbeing* occurs 5 times in the *Principles*, in contrast to scores of uses of the terms *inhumanity* and *distress*. One occurrence is in a mention of studies that create nutritional deficiencies in animals: “The mildest of such symptoms is a

general decline in weight and wellbeing; often quite specific and almost certainly distressing pathological states are produced, such as polyneuritis or rickets" (p 98).¹⁶ In this passage, decline in wellbeing is regarded as a sign of distress, which Russell and Burch go on to suggest might be prevented in such nutritional studies through use of microorganisms (p 98). Another passage characterizes grooming and comfort behavior in certain species as "(i)ndices of wellbeing" (p 28).¹⁶ In this discussion, absence of wellbeing is also presented as a sign of distress, something entirely consistent with Russell and Burch's characterization of the 3Rs as ways of minimizing distress.

It is in the remaining 3 uses of the term *wellbeing* that Russell and Burch might suggest that wellbeing, and not diminution and elimination of distress, is the fundamental aim of the 3Rs. The 2 paragraphs in question occur immediately before the passage we quote earlier, in which Russell and Burch characterize distress (inhumanity) and its measurement in terms of a scale or spectrum. In that passage, distress or inhumanity is at one end of the scale and the absence of distress (humanity) is at the other. In that passage, the aim of the new science of humane experimental technique appears to be the progressive reduction of distress until absence of distress (humanity) is reached at the other end of the scale.

After their initial discussion of pain, fear, and conflict as kinds of distress, Russell and Burch remark that "(i)n mammals at least, there is some ground for postulating a convenient linear polarization of behavioral states, along a spectrum extending from complete wellbeing to acute distress" (p 22).¹⁶ They discuss several physiologic components of "such general components as approach and avoidance." (p 22) They continue:

Now persistent activation of the mass sympathetic response is liable eventually to merge into the much more catastrophic stress syndrome associated with the adrenal cortex and other endocrines. [reference omitted] *We can therefore begin tentatively to think of a scale of wellbeing to distress, linked with a scale of relative predominance of the two autonomic modes of activity. So closely are the two scales linked, in fact, that the term "emotional mechanism" is often used in the literature (for example in characterizing brain regions) in a sense which turns out to mean simply the presence of exaggerated autonomic effects. There has been a curious tendency here to connect the term 'emotion' specifically with the emergency or distress end of the scale, as though emotions were always unpleasant. But we need not restrict ourselves to either end, or to exaggerated effects. All variations in mood with perceptibly different behavioral outcomes must also have perceptibly different autonomic effects. We can feel fairly confident that along the whole spectrum the two scales are kept perfectly in line, probably by such special mammalian integrating mechanisms as the hypothalamus and rhinencephalon. It may be more satisfactory to think in terms of a scale than of two poles. In this way we are led to set our sights high in removing inhumanity, and to attempt always to drive the animal up to the highest possible point on the scale. Thus, we can aim at wellbeing rather than at mere absence of distress. Everything we know of the phenomena of suggestion [reference omitted] is in favor of such a policy (p 23, italics added).*¹⁶

In this passage, Russell and Burch repeat the principle that occurs throughout their book, that the aim of the new science of humane experimental technique is the removal of inhumanity and the achieving of humanity. However, in *this* passage humanity appears to be not absence of distress—as it is characterized in the passage describing distress and its measurement, and elsewhere in the *Principles*—but *more* than "mere absence of distress." In this passage, humanity appears to be wellbeing, or as Russell and Burch put it preceding the paragraph quoted earlier, "complete wellbeing." As indicated in the first italicized sentence, the scale or spectrum presented in the discussion of wellbeing has distress at one end and wellbeing (or complete wellbeing) at the other. The second italicized sentence appears to indicate that this latter end of the scale is humanity, because as one proceeds up this scale, one is progressively decreasing inhumanity. In other words, in *this* scale, *humanity* appears to mean wellbeing or complete wellbeing.

In sum, the *Principles* appears to present in close proximity 2 different scales and general conceptions of the aim of the new science of humane experimental technique. One scale has distress at one end and humanity (understood as absence of distress) at the other, with the aim of achieving humanity understood as lack of distress. The other scale has distress at one end and wellbeing or complete wellbeing at the other, with the aim of achieving humanity understood as such wellbeing.

Different possible interpretations of the discussion of wellbeing. The first problem one faces in attempting to understand their discussion of wellbeing is that Russell and Burch do not define *wellbeing* and do not describe feelings of wellbeing, aside from characterizing them as "emotions" that are not "unpleasant." However they understand *wellbeing*, there are several possible interpretations of the 2 different scales presented in the *Principles*, and their possibly different conceptions of the aim of the 3Rs in removing inhumanity.

One interpretation, which would accord with much contemporary thinking about the removal of distress in laboratory animals (including, as we shall illustrate, some statements about the 3Rs Russell and Burch made after publication of the *Principles*) is that an effective way of diminishing and removing distress is sometimes to promote conditions in which animals are comfortable and experience wellbeing in some sense. The problem with this interpretation is that their discussion of wellbeing seems to postulate wellbeing and not merely absence of distress as the *aim* of the new science and of the 3Rs.

Perhaps what Russell and Burch mean in the discussion of wellbeing is that humanity is, as elsewhere in the *Principles*, the absence of distress, but that when humanity so understood is reached—through diminution or removal of inhumanity accomplished by use of the 3Rs—a state of wellbeing results. The plausibility of this interpretation depends on how *wellbeing* is defined. When distressful feelings of pain or fear, for example, are lessened some animals may well feel a sense of relief. However, this would not seem to constitute *complete wellbeing*. If *complete wellbeing* means positive experiences of comfort, pleasure, or happiness, it seems patently incorrect to assert that wellbeing always will occur simply upon the elimination of distress. However, even if wellbeing in some sense just results from diminishing or removing distress, in their discussion of wellbeing Russell and Burch still appear to suggest that their new science *aims* at wellbeing—and not that wellbeing is a fortunate result of the absence of distress.

A third possible interpretation of the discussion of wellbeing is that *humanity* (understood as absence of distress) is *not* the ultimate goal of the new applied science and of the 3Rs.

On this interpretation, the scale Russell and Burch think is the more fundamental of the 2 scales is the scale with distress at one end and wellbeing (or complete wellbeing) at the other. The scale with distress at one end and humanity (understood as absence of distress) at the other end would constitute only the first part of the former scale. That is, as one moves away from extreme distress or inhumanity, one reaches (perhaps at the midpoint of this scale) lack of distress or humanity, and then as one moves further up the scale one eventually reaches wellbeing or complete wellbeing. On this interpretation of the passage discussing wellbeing, Russell and Burch would view the reduction and elimination of distress and inhumanity (and use of the 3Rs) as just part of the program of the new science of humane experimental technique, to be followed by use and development of techniques for promoting wellbeing. However, nowhere else in the *Principles* do Russell and Burch indicate that the 3Rs and their aim of minimizing distress constitute only part of the endeavor of achieving humane treatment of research animals.

Yet another possible interpretation of the discussion of wellbeing is that humanity remains the ultimate aim of the 3Rs—and occupies the end of the scale of progression toward humane animal use opposite from distress—but that *humanity* does not mean absence of distress, but wellbeing or complete wellbeing. This interpretation too contradicts the clear and repeated statements and extended discussions in the *Principles* indicating that humanity is indeed the absence of inhumanity or distress.

Later statements about wellbeing. Decades after publication of the *Principles*, Russell and Burch made several statements about wellbeing and the 3Rs. Most of these statements seem to endorse promoting wellbeing as a means of effecting reduction and refinement and thereby reducing distress. In a 1999 lecture Russell referred to studies of laboratory animals by the ethologist Michael Chance, who “made the very important discovery that conditions favouring their wellbeing make animals more physiologically uniform, so that smaller samples are needed for experiment” (p 277).¹⁵ Here, Russell appears to view promotion of wellbeing simply as a method of effecting reduction, which is presented in the *Principles* as a way of minimizing distress. In a 2002 discussion, citing the work of Chance and others, Russell argued that contingent inhumanity and distress can be lessened, and experimental results maximized, through “the provision of comfortable quarters—including handling procedures—with a stable environment, companionship, and freedom to engage in species-typical basic activities” (p 3).¹³ In a 1995 review of the progress of the 3Rs after publication of the *Principles*, Burch appears to have reiterated the view that making animals comfortable, through appropriate transport, handling, restraint and care, can reduce distress. He also stated that Chance’s work on wellbeing and sample size

and other evidence considered in our book, strongly suggested that conditions optimal for uniformity *are also optimal* for the health, wellbeing and comfort of the animals, which also, of course, means optimal for their performance in experiments, for the disturbing effects of even mild distress on experimental results is one aspect of one of the main themes of our book, namely the close relationship between humaneness and the scientific value of experiments. Here, then, in the control of environmental conditions during both rearing and testing, *reduction* and *refinement* go hand in hand (p 273, italics in original).⁷

Burch appears to say in this passage that wellbeing and comfort enable uniformity and use of fewer animals because they remove the “disturbing effects” of distress, indeed even of mild distress. This view is consistent with the definition of refinement in the *Principles* as ways of reducing distress. In a 2005 publication, Russell appears to repeat the view that providing comfort and wellbeing can achieve reduction and refinement as defined in the *Principles*. “Hitherto,” he states, “it had always been supposed that to make animals uniform it was only necessary to keep them in the same environment. Chance discovered that *some environments are more favorable to uniformity than others*. The most uniform populations of all were those kept in *an environment optimal for their wellbeing*. In this respect, the goal of reduction is precisely the same as the goal of *refinement*” (p 283, italics in original).¹⁴

Unfortunately (for the interests of clarity), Russell continues after the passage quoted immediately above as follows:

We originally envisaged refinement as minimizing pain and distress, and by 1959, discomfort.¹¹ It is now clear that we must aim positively at optimal wellbeing, for the following reason. “...the major discovery of anatomy and physiology in the last half-century has been that of the extraordinarily subtle, comprehensive and intimate linkages and interactions between the somatic nervous system, the organ of behavior, and the autonomic nervous system and the endocrine system, which control events within the body” (p 9).¹⁶ It was already clear in the 1950s which parts of the brain were chiefly involved in these linkages—the hypothalamus in all vertebrates and the limbic system in mammals. These connections are capable of “converting distress caused by the physical, behavioural or social environment into physiological stress bound to disturb experimental results.... More is known now about the pathways to and from the limbic system, and the corticotropin-releasing factor in the hypothalamus (discovered in 1955) was isolated in 1981 and has since been the subject of numerous studies—some in vitro— and related substances have been found in lower vertebrates.”¹² In man, this is the basis for the discipline of psychosomatic medicine, which is equally important in the veterinary context (p 283).¹⁴

In the opening 2 sentences Russell appears to be saying that he and Burch came to *reject* the definition of refinement in the *Principles* as ways of minimizing distress and subsequently defined refinement as aiming at optimal wellbeing. It is not clear, however, that this is what Russell really means. First, the scientific evidence Russell offers for the supposed *change* from minimizing pain and distress to promotion of wellbeing relates to causes and amelioration of *distress*. Russell speaks about neural and physiologic connections that convert distress into stress that confounds experimental results. Moreover, the corticotropin-releasing factor that he suggests is enhanced by comfort and wellbeing counteracts *distressful* states such as anxiety and depression. Closer examination of the first 2 sentences of the passage reveals additional evidence that Russell might not have intended to suggest an alternate definition of refinement. By citing in the first sentence a 1959 article other than the *Principles*, Russell appears to say that as early as 1959, the original definition of refinement in their book had already been superseded by a

new definition that includes minimizing discomfort. However, in 1995, Russell and Burch were defining refinement as “refinement of procedures to keep to an absolute minimum the distress imposed on animals still used for experiment” (p 267).¹⁷ In a 1999 lecture, Russell defined refinement as attempts “to reduce distress to a minimum and, by the same token, avoid physiological disturbances that would upset the experimental results” (p 277).¹⁵ Even more significant is Russell’s 2002 discussion of the role of comfortable quarters and good handling in reducing and eliminating contingent inhumanity and improving experimental results. Russell concludes that from the substantial evidence of the relationship between comfort and wellbeing and improved scientific results, “(i)t follows that the third ‘R’—Refinement—is concerned not only with minimizing distress during experiments (e.g., by the use of analgesics) but with maximizing comfort and wellbeing of the animals in husbandry” (p 1).¹³ However, in the paragraph preceding this statement, Russell *defines* refinement as “refinement of procedures actually used to minimize the distress imposed on the animals.” (p 1) This indicates that in saying that refinement is also concerned “with maximizing comfort and wellbeing of the animals in husbandry,” and in asserting in the longer quotation above that it is “now” clear that we must aim positively at optimal wellbeing, Russell merely means to say that if one wants to minimize distress it is sometimes better not to focus merely on feelings of distress and how these feelings might be reduced. Rather, Russell may be saying, promoting wellbeing or optimal wellbeing often provides the most effective way of minimizing distress. If this is what Russell means, he is not endorsing an alternate definition of refinement in which the aim of refinement is wellbeing instead of or in addition to minimization of distress. He is simply maintaining that promoting wellbeing is an important form of refinement as it has always been defined in the *Principles* as “any decrease in the incidence or severity of inhumane procedures applied to those animals which still have to be used” (p 64).¹⁶

Upshot for understanding refinement and the aim of the 3Rs.

We submit that when one considers the *Principles* as a whole, and statements Russell and Burch subsequently made about wellbeing and the 3Rs, it is more likely that they did not depart from the definition of refinement in the *Principles*, and from their view of the general aim of the 3Rs, as reducing and eliminating distress. It is also significant that the discussion of wellbeing in the *Principles* occurs before the first mention and definitions of the 3Rs, including refinement. This also appears to support the conclusion that when it came time actually to *define* refinement, Russell and Burch did not intend to include promoting wellbeing in this definition or in the general aims of the 3Rs. However, if Russell and Burch did sometimes have in mind an alternate definition of refinement, their discussion of refinement in such an alternate sense provides no assistance to those who might also want to define refinement to include wellbeing. Russell and Burch do not define or characterize in any detail the nature of *wellbeing*, *complete wellbeing*, or *optimal wellbeing*. They do not provide arguments for the view that investigators are ethically obligated to provide animals wellbeing as well as freedom from unnecessary distress. They do not address any of the questions we raise below regarding the advisability of requiring investigators to provide animals wellbeing in some sense. For all these reasons, we now consider the definitions of the 3Rs that clearly *are* offered in the *Principles*.

Replacement

The definition in the *Principles*. As noted earlier, in introducing the 3Rs, Russell and Burch define replacement

as “the substitution for conscious living higher animals of insentient material” (p 64).¹⁶ At the opening of their later chapter on replacement, they provide a somewhat more detailed definition:

We shall use the term ‘replacement technique’ for any scientific method employing non-sentient material which may in the history of experimentation replace methods which use conscious living vertebrates. Among this non-sentient material, we include higher plants, microorganisms, and the more degenerate metazoan endoparasites, in which nervous and sensory systems are almost atrophied (p 69).¹⁶

Importantly, replacement is *not* defined in the *Principles* as the use of nonanimal material instead of animals. Replacement is defined as the use of *insentient* (or nonsentient) material instead of sentient material. Russell and Burch do not define replacement as not using animals because they classify *the use of insentient animals as instances of replacement*. They distinguish between what they call *relative* and *absolute* replacement.

In relative replacement, animals are still required, though in actual experiment they are exposed, probably or certainly, to no distress at all. In absolute replacement, animals are not required at all at any stage. It follows from what has been said earlier that absolute replacement may be regarded as the absolute ideal (p 70).¹⁶

Russell and Burch do not suggest that they view absolute replacement as preferable to relative replacement because they think that not using animals is in and of itself preferable from a scientific or ethical standpoint to using animals. When they speak of “what has been said earlier,” they are simply referring to their earlier characterizations of the aim of replacement (and all 3Rs) to diminish and whenever possible remove distress. They consider absolute replacement to be the absolute ideal because when no animal is used there is *absolutely* no chance that an animal will experience *distress*. That the primary aim of replacement is elimination of distress and not elimination of the use of animals is also clear from their discussion of 2 examples of relative replacement.

First, there is the case of nonrecovery experiments on living and intact but completely anesthetized animals. Provided the anesthesia is general and sufficiently deep, and its time-course properly synchronized with the treatment itself, such treatments are totally free from inhumanity. The qualification is of course, important [citation omitted], in relation to contingent inhumanity. Provided the qualification is met, even recovery experiments may fairly be included in this category if they involve, for instance, the injection of a drug with transient effect which does not outlast the anesthesia.

Second, we may consider experiments in which animals are still required, but only to furnish preparations after being painlessly killed. This already constitutes a further advance. Provided the euthanasia is satisfactory, and provided there is substantial reduction in numbers, such experiments are beyond reproach (p 71).¹⁶

Departures from the *Principles*: replacement as not using animals or using less-sentient animals. Many recent definitions of replacement that claim to follow the *Principles* define replacement as not using animals, not using vertebrate animals, or using less-sentient animals. The American Veterinary Medical Association Policy on the Use of Animals in Research, Testing, and Education (AVMA Policy) states that “the AVMA endorses the principles embodied in the ‘3R’ tenet of Russell and Burch (1959),” and defines replacement as “replacement of animals with nonanimal methods wherever feasible.”² The Institute of Laboratory Animal Research (ILAR) *Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research* (ILAR Guidelines for Neuroscience Research) attributes to the *Principles* the definition of replacement as “(u)se of nonanimal systems or less-sentient animal species to partially or fully replace animals” (p 10).⁸ According to the ILAR *Report on the Recognition and Alleviation of Distress in Laboratory Animals* (ILAR Report on Distress), the *Principles* defines replacement as “replacement of an animal with a nonanimal model or a less sentient species, usually of a lower phylogenetic order, such as a primitive invertebrate” (p 64).⁹ APHIS Policy 12 claims that in the *Principles* replacement is defined as the use of “nonanimal systems or less-sentient animal species to partially or fully replace animals (for example, the use of an in vitro or insect model to replace a mammalian model).”² The ILAR *Guide for the Care and Use of Laboratory Animals (Guide)* asserts that in the *Principles* replacement “refers to methods that avoid using animals. The term includes absolute replacements (i.e., replacing animals with inanimate systems such as computer programs) as well as relative replacements (i.e., replacing animals such as vertebrates with animals that are lower on the phylogenetic scale)” (p 5).¹⁰

Some of these definitions are unclear. For example, the definition in the *Guide* does not indicate what it means by replacing animals “such as vertebrates.” Nor does this definition indicate whether “relative replacement” means replacing any vertebrate with a nonvertebrate species or replacing a vertebrate species with any species, vertebrate or nonvertebrate, lower on the phylogenetic scale (for example, replacing monkeys with mice). The definition in APHIS Policy 12 does not appear to restrict animals used as replacements to nonvertebrates, as long as the species used is “less sentient.” However, this definition (like the definition in the ILAR Report on Distress) does not indicate what it means by “less sentient,” which could denote not being able to experience any pain or distress or being able to experience some pain or distress but of a level not as severe as that which can be experienced by other species.

In any event, none of the definitions of replacement quoted above faithfully follows the definition in the *Principles*. The *Principles* never defines replacement as not using animals or using animals that feel less distress than vertebrates or some vertebrates. Replacement means using *completely insentient* material, animal or nonanimal. Moreover, using animals that are *less sentient* in the sense of feeling less distress than can be experienced by vertebrates or some vertebrates not only has nothing to do with replacement as Russell and Burch define it but is inconsistent with their definition. The examples they provide of animal species that can be used in replacement techniques (“the more degenerate metazoan endoparasites”) are included because Russell and Burch believe that they are completely nonsentient, not because they are *less sentient*. Nor can Russell and Burch be interpreted to recommend or countenance use of *lower* sentient vertebrate species that are *less sentient* than other vertebrates. They explicitly argue that, because of more limited mental capacities that prevent them from understanding and dealing with distressful experiences, for lower

vertebrates, a given level of distress is probably worse than it is for a higher vertebrate species. “In general,” they say,

the lower animal is the slave of its own moods. Its behavior is very largely automatic, and we know that we ourselves are most vulnerable when our behavior is most automatic. ... Far from despising lower animals (as it is convenient to call them) for these deficiencies, we should logically treat them with special consideration (17).¹⁶

Departures from the *Principles*: replacement as a goal irrespective of its ability to diminish or remove distress. Another common departure from the definition of *replacement* in the *Principles* is failing to identify as the aim of replacement the diminution or elimination of distress—but instead presenting replacement as a goal separate from the goal of minimizing distress. This latter approach typically is accomplished by providing simultaneous definitions of all 3Rs and by including reduction or elimination of pain or distress *only* in the definition of refinement. Examples of such definitions of replacement can be found in the definitions of the 3Rs in the AVMA Policy and the *Guide*, quoted earlier. In contrast, APHIS Policy 12, after citing the *Principles* in support of its definitions of the 3Rs states that

(a)lternatives or alternative methods, as first described by Russell and Burch in 1959, are generally regarded as those that incorporate some aspect of replacement, reduction, or refinement of animal use in pursuit of the minimization of animal pain and distress consistent with the goals of the research.²

The APHIS Policy 12 definition of replacement (as well as of reduction and refinement) thus follows Russell and Burch in stipulating that by their very nature all of the 3Rs seek to minimize animal pain and distress consistent with project goals. The ILAR Report on Distress also states that “(t)he simplest approach to avoiding, minimizing, and alleviating distress in laboratory animal care and use is to follow the principles of the 3Rs—refinement, reduction, and replacement” (p 63).⁹

Questions raised by and potential consequences of modified definitions of replacement. Russell and Burch define replacement as the use of insentient material because of their focus on minimizing distress. Although virtually all more recent definitions define replacement as use of nonanimal material, one aim even of such definitions can still be minimization of distress. However, as we have noted, some of these definitions appear to reflect an underlying view it is preferable not to use animals in research—not just because avoiding animals can diminish or eliminate distress, but for some other reason or reasons.

Such definitions of replacement typically are just stated, without supporting argument, and attributed to the *Principles*. Explicit and clear reasons are not provided for *why* using no animals in a given experiment or animal research generally would be better than using any animals—irrespective of the ability of nonanimal materials to eliminate distress. It is apparently supposed to be self-evident that it is preferable to use no animals, even if animals that are used experience *no* pain or distress or, indeed, have a much better life than they would in natural environments. However, different arguments can be raised for avoiding the use of animals in research, irrespective of or in addition to the importance of sparing animals unnecessary distress.²⁰ It can be maintained, for example, that using no animals would reduce the expense of research, and that this would be a good thing in itself or might allow more research

using the same financial resources. It can be argued that using animals in research can be difficult, time-consuming, or inefficient. It can be argued that eliminating animal use would be preferred by the public and thus would enhance public support for biomedical research.

Adoption of definitions of replacement that depart from the definition in the *Principles* can have significant consequences. For example, if replacement is defined to include use of *less-sentient* animals in a sense that nevertheless allows these animals to experience distress, investigators who use such animals instead of another *more-sentient* species will accomplish some measure of replacement. However, this would *not* constitute replacement as defined in the *Principles*. Investigators who follow Russell and Burch's definition will successfully practice replacement if they use insentient animals. However, these same investigators would *fail* to practice replacement if, by definition, replacement requires not using animals. And if the aim of replacement so defined includes such things as reducing the economic cost or assuring public support of research, to show that they have incorporated replacement for the right reasons, investigators will have to be able to demonstrate that using no animals in given experiments is in fact supported by these reasons. This may not always be easy or possible. In contrast, for Russell and Burch, if an investigator uses *insentient* materials, animal or nonanimal, replacement is accomplished, and for a clearly demonstrable reason: *no distress* is experienced.

Reduction

The definition in the *Principles*. The *Principles* defines reduction as "reduction in the numbers of animals used to obtain information of a given amount and precision" (p 64)¹⁶ Like the other Rs, reduction serves the aim of reducing and when possible removing inhumanity or distress.

Reduction not defined as minimizing or attempting to minimize numbers. The *Principles* does not define reduction as the *minimization* of the number of animals used to obtain information of a given amount and precision. Reduction is defined simply as *reduction*—which is not synonymous with *minimization*. Russell and Burch do not explain why, given that they aim at minimizing animal distress, they do not define reduction as minimization of animal numbers. However, if reduction were so defined, in a given experiment or kind of research, there would be *no* reduction unless the absolutely smallest number of animals to achieve a given result is used. Russell and Burch emphasize that it is often impossible to know before an experiment is conducted whether the minimal number of animals will be used. Discussing the importance of use of statistical methods in reduction they state that

For reduction purposes, as we have noted, the statistical method has a key property—it specifies the minimum number of animals needed for an experiment. This statement needs qualification. It certainly is always possible, in accordance with the arbitrary but workable concept of significance level, to decide *after the event* whether enough animals have been used. This saves needless repetition, and where, as sometimes in bioassay, workers are familiar with the amount of variation to be expected, a number found to give significant results can be fixed upon for regular practice. Exact treatments of the problem of choosing the right number *in advance* on the basis of experience are limited in scope so far (p 111, italics in original).¹⁶

Russell and Burch clearly want scientists to practice reduction *now*, so that the number of animals can at least be reduced progressively as statistical and experimental techniques are improved. Defining reduction as achieving absolute minimization of numbers of animals used would be inconsistent with this aim.

In light of the passage just quoted—and many discussions in the *Principles* in which techniques for reducing numbers of animals are urged—some might think that Russell and Burch surely *mean* by reduction *attempting* to reduce to the absolute minimum the number of animals used to obtain information of a given amount and precision. Although they do not indicate why they do not so define reduction, doing so would be unwise in light of what they say about the aim of the new science of humane experimental technique. As noted earlier, an essential goal of this science is the conduct of scientifically sound and successful experimentation and testing. Aiming at absolute minimization rather than reduction might deter some investigators from conducting sound science by using too few animals, and as a result, subject animals that are used to pointless distress. In this regard, it should be noted that in the passage just quoted, in speaking about the ability of statistical analysis to indicate the *minimum* of animals needed in an experiment, Russell and Burch state that the aim is not just to avoid using too many animals, but also to assure, to quote them exactly, that "*enough* animals have been used." Russell and Burch clearly do not want reduction to result in the use of too few animals.

A second good reason for Russell and Burch not to include attempting to minimize the number of animals in the definition of reduction relates to our earlier point regarding possible conflict between the aims of minimizing distress experienced by individual animals in an experiment and of minimizing the sum total of distress experienced by all the animals. In an experiment that inflicts some pain or distress, reducing to—or even aiming at—the *absolute minimum* of animals that are needed might in some cases involve subjecting individual animals to a great deal more distress. This approach might not always be the most humane.

Departures from the *Principles*: minimization and other changes. Some recent definitions of reduction erroneously attributed to the *Principles* define reduction as absolute minimization of animal numbers. For example, the ILAR Report on Distress states that the *Principles* defines reduction as "reduction of the number of animals used to the absolute minimum necessary (based on appropriate statistical sample size determination or other field-specific methods), particularly if they are likely to experience unavoidable distress" (p 64).⁹ APHIS Policy 12 attributes to the *Principles* the definition of reduction as "methods that reduce the number of animals to the minimum required to obtain scientifically valid data."² The ILAR Guidelines on Neuroscience Research claims that the *Principles* defines reduction as "reduction in the number of animals used to the minimum required to obtain scientifically valid data" (p 10).⁸

In contrast, the AVMA Policy defines reduction as "reduction of the number of animals consistent with sound experimental design."³ Like the definition in the *Principles*, this definition does not include minimization or attempted minimization of animal numbers. According to the *Guide*, "(r)eduction involves *strategies for obtaining comparable levels of information from the use of fewer animals* or for maximizing the information obtained from a given number of animals (without increasing pain or distress) so that in the long run fewer animals are needed to acquire the same scientific information" (p 5, italics added).¹⁰ The italicized portion of this definition reflects in substance the definition in the *Principles*.

Departures from the *Principles*: reduction as a goal irrespective of its ability to diminish or remove distress. As is the case with replacement, some recent definitions of reduction attributed to the *Principles* fail to indicate that, for Russell and Burch, reduction aims by its nature at diminishing and, when possible, eliminating inhumanity or distress. As is the case with replacement, this modification in the definition of reduction is accomplished by including diminution or elimination of distress only in the definition of refinement. The AVMA Policy presents reduction in this manner,³ as does the *Guide's* definition (p 5).¹⁰ Whatever this latter definition means by including within reduction “maximizing the information obtained from a given number of animals (without increasing pain or distress) so that in the long run fewer animals are needed to acquire the same scientific information” (p 5), this definition does not indicate that (as in the *Principles*) the sole aim of reduction is diminution or elimination of distress. Although these words counsel investigators not to allow reduction to result in additional pain or distress, the *Guide's* definition of reduction still appears to present using fewer animals as an aim that is separate from the minimization of distress.

Questions raised by and potential consequences of modified definitions of reduction. Definitions that characterize reduction as an aim separate from the aim of reduction or elimination of distress reflect an underlying view that it is preferable to use fewer rather than more animals where possible—not because or not just because using fewer animals can reduce distress—but because it is preferable to use fewer animals for some other reason or reasons. As is the case with definitions of replacement that depart from the definition in the *Principles*, modified definitions of reduction typically are not accompanied by *arguments* supporting the view that it is better, irrespective of effects on animal distress, to use fewer rather than more animals. It is incumbent on supporters of such definitions to provide such arguments. Perhaps some proponents of such modified definitions of reduction believe that there is something inherently and unavoidably wrong in using animals in research—and that when it is necessary to use animals (a ‘necessary evil’ in such persons’ view), using fewer animals is therefore better (a ‘lesser evil’) than is using more animals. Perhaps some proponents of such modified definitions of reduction would defend these definitions by pointing to potential savings in cost of research, difficulties and inconveniences in using animals in research, or opposition to animal use by some members of the public.

Adoption of definitions of reduction that depart from the definition in the *Principles* can have significant consequences. For example, if reduction is defined as absolute minimization of numbers of animals, it will sometimes—perhaps often—be difficult to know that reduction has been accomplished at the time of an experiment. Investigators who follow a definition that does not tie reduction necessarily to the goal of reducing or eliminating distress would fully accomplish the aim of reduction simply by reducing the number of animals used in an experiment, even if this would result in significantly *more* distress experienced by individual animals or more total distress experienced by all the animals. And if the aim of reduction is something other than reduction or elimination of animal distress, such as reducing the economic cost or assuring public support of research projects, to show that they have applied reduction for appropriate reasons, investigators will have to be able to demonstrate that reducing the number of animals in a given experiment is in fact supported by these reasons. In contrast, investigators who follow the definition of reduction in the *Principles* will be able to focus strictly on the issue of how

reducing the number of animals might reduce any *distress* the animals might experience.

Refinement

The definition in the *Principles*. The *Principles* defines refinement as “any decrease in the incidence or severity of inhumane procedures applied to those animals which still have to be used” (p 64).¹⁶ Russell and Burch subsequently state that the “object [of refinement] is simply to reduce to an absolute minimum the amount of distress imposed on those animals that are still used” (p 134).¹⁶ They do not explain why, although the object of refinement is the absolute minimization of distress, this object is not included in the *Principles' definition* of refinement. However, defining refinement as absolute minimization of inhumanity or distress would imply that investigators who fail to absolutely minimize distress achieve *no* refinement. Defining refinement as *any* diminution of inhumanity conceives of refinement as an approach that can be used even when it may not be possible to know that distress will be absolutely minimized.

It must be noted, however, Russell and Burch sometimes did seem to define refinement in terms of absolute minimization. In 1995, they characterized refinement as “procedures to keep to an absolute minimum the distress imposed on animals still used for experiment” (p 267).¹⁷ In 1999, Russell described refinement as “procedures when animals are still used, to reduce distress to a minimum” (p 277).¹⁵ In 2002, Russell characterized refinement as “procedures actually used to minimize the distress imposed on the animals” (p 1).¹³ The original definition in the *Principles* is, we submit, better suited to express not only the ultimate aim of refinement (and of all 3Rs) to minimize distress, but also the reality that this aim cannot always be achieved in every animal research project.

Possible departures from the *Principles*: pain and distress compared with distress. The *Principles* defines refinement as any diminution of inhumanity (understood as distress). In contrast, many current definitions of refinement attribute to the *Principles* a definition that speaks of refinement as the reduction or elimination of *pain and distress* or of *pain or distress*. For example, the AVMA Policy characterizes the definition of refinement in the *Principles* as “refinement of experimental methods to eliminate or reduce animal pain and distress.”³ The *Guide* states that in the *Principles* refinement aims by definition to “minimize or eliminate pain and distress” (p 5).¹⁰ APHIS Policy 12 speaks of the 3Rs as “methods that refine animal use by lessening or eliminating pain or distress.”²

The definition of refinement in the *Principles* does not speak of diminution or removal of pain *and* distress (or of pain *or* distress) because Russell and Burch classify pain as one form of distress. It is beyond the scope of this article to consider whether government authorities and IACUCs in the United States in fact interpret the term “distress” to include all the mental states included in this term in the *Principles*. If the ethical aim of humane use and care of research animals includes the goal of sparing them from *all* significantly unpleasant feelings that are not necessary for the purposes of research, a broad definition of *distress* seems advisable. The *Principles*, we submit, makes a very strong case for including in the definition of *pain and distress* mental states such as fear, anxiety, boredom, hunger, thirst, bodily discomfort, and any other significantly unpleasant feelings.

Departures from the *Principles*: enhancement of wellbeing. Some widely used definitions follow the *Principles* in restricting *refinement* to reduction or minimization and prevention of pain and distress. The AVMA Policy, for example, states that the *Principles* defines refinement as use of “experimental methods

to eliminate, or reduce animal pain and distress.^{7,2} The ILAR Report on Distress attributes to Russell and Burch the definition of refinement as “refinement of the protocol to minimize or eradicate distress for the species used” (p 64).⁹

However, several definitions add to the definition of refinement (and attribute to the *Principles*) methods that enhance animal *wellbeing*. The ILAR Guidelines on Neuroscience Research defines refinement as “(u)se of a method that lessens or eliminates pain and/or distress and therefore enhances animal wellbeing” (p 10).⁸ According to APHIS Policy 12, as defined in the *Principles*, *refinement* refers to “methods that refine animal use by lessening or eliminating pain or distress and, thereby, enhancing animal wellbeing (for example, the use of appropriate anesthetic drugs).”² The *Guide* attributes to Russell and Burch the definition of refinement as “modifications of husbandry or experimental procedures to enhance animal wellbeing and minimize or eliminate pain and distress.” (p 5).¹⁰

What is wellbeing? It is not clear what any of these definitions mean by *wellbeing* or whether they all use this term in the same sense. As we noted earlier, the term *wellbeing* can refer to feelings ranging from absence of distress to happiness. It is possible to interpret the definitions in the ILAR Guidelines on Neuroscience Research and APHIS Policy 12 to mean by *wellbeing* simply the absence of pain or distress—because these definitions assert that minimizing pain or distress “therefore” or “thereby” results in enhancement of wellbeing. The statement in Policy 12 that wellbeing is enhanced by use of anesthetic drugs supports this interpretation, because all that results from anesthesia is freedom from pain—and not wellbeing in some positive sense. In contrast, these definitions might intend to say that minimizing pain or distress results in *something else*, namely wellbeing. If *wellbeing* is intended in these definitions to mean more than the absence of pain or distress, neither definition of refinement—or any discussions accompanying them—indicate what such *wellbeing* is. In defining refinement as modifications to “enhance animal wellbeing *and* minimize or eliminate pain and distress,” (p 5, italics added)¹⁰ the *Guide* appears to conceive of wellbeing as more than minimization or elimination of pain and distress. However, the *Guide* never defines the term *wellbeing*.

Questions raised by and potential consequences of modified definitions of refinement. The major departure from the definition of refinement in the *Principles* is the addition of the promotion of wellbeing to minimization of distress.

What is wellbeing? It is essential that proponents of such definitions indicate clearly—as none has yet done—what the term *wellbeing* means. Without such clarity, it is impossible to know what these definitions of refinement call upon investigators and IACUC to provide to research animals. The *Guide* contains extensive discussions of housing conditions that allow satisfaction of basic needs and certain species-specific behavior and of techniques to promote environmental enrichment. Such measures may well promote wellbeing in some sense; however, they do not *define* what wellbeing is. Only, we submit, if we *begin* (as did Russell and Burch in their formal definitions of the 3Rs) with a clear idea of what animals should or should not experience, can we then set about finding ways to promote or prevent such experiences. The task of defining *wellbeing*—and asking investigators to provide refinement that includes promoting it—may not be easy, if wellbeing is supposed to include such mental states as *pleasure, contentment, or happiness*. These latter terms also need to be defined as they apply to research animals. It must be established whether and when it can confidently be said that research animals of various species experience such mental states, and how investigators can provide them to various species.

A clear definition of *wellbeing* (if it is to be included in the definition of refinement) is also necessary in fairness to investigators and research facilities. For without such a definition, some investigators who would seek to make their animals very happy (if this how they understand *wellbeing*) might incur significant expense and effort, and thus might compromise their ability to pursue the scientific goals of some experiments—expense and effort that would not be incurred by investigators who aim lower, say, at providing only slight comfort or a few modest pleasures (if this is what *they* think constitutes *wellbeing*).

Why should research animals be provided wellbeing? Those who endorse definitions of refinement that include enhancement of wellbeing must be clear (as admittedly Russell and Burch themselves might not have been) whether providing wellbeing is solely a means of diminishing or eliminating distress, or whether wellbeing is due to animals for other reasons. This distinction has important practical consequences. If wellbeing should be provided only because it can reduce distress, wellbeing would need to be provided only when and to the extent to which it fulfills this aim. If, in contrast, research animals are owed wellbeing independently of the need to spare them unnecessary distress, investigators and facilities will need to provide wellbeing to animals in many more kinds of experiments (for example, when, as is often the case, animals experience no pain or distress). It might seem ungenerous to question whether research animals should be happy or experience pleasures (if this is what the term *wellbeing* means). However, the prevailing view in our society and legal system (as expressed in state cruelty to animals laws and the federal Animal Welfare Act, for example) is that animals used for human benefit should not be subjected to unnecessary or unjustifiable pain or distress, not that they are entitled to pleasure, contentment, or happy lives.¹⁹ In light of the potential practical consequences of aiming at wellbeing irrespective of minimization of distress, those who believe that research animals are entitled to pleasure or happiness (if this is what they mean by *wellbeing*) through the definition of refinement, surely should indicate *why* they think this is so.

What effects will providing wellbeing have on research? The *Principles* insists that the 3Rs be used “without prejudice to scientific and medical aims” (p 14).¹⁶ This condition can be met even if refinement is understood as including enhancement of wellbeing—provided that investigators are not asked to enhance wellbeing if doing so could confound experimental goals or results. Nevertheless, it is reasonable to ask whether definitions of *refinement* that include wellbeing as an aim separate from reduction or elimination of distress could still affect research goals indirectly. For example, would providing pleasures or happy lives for research animals (assuming that *wellbeing* includes such things and that we know how to provide them to animals) hinder or sometimes preclude valuable research by increasing its economic costs? Will the research community need to expend resources and efforts that could be devoted directly to research projects on studies aimed at discovering how various kinds of wellbeing can be afforded to various species in various kinds of research that use various scientific procedures? It might be unwise to adopt definitions of refinement that might have such effects on research, at least without sustained consideration of whether these effects would occur and whether and to what extent they are acceptable.

Should promotion of wellbeing be included in the definition of refinement, or should it be considered and dealt with separately? It may well be that the aims of doing what is good for research

animals and advancing the quality of research and testing are served by promotion of wellbeing in some sense. It may be that promoting wellbeing is often an effective tool in minimizing research animal distress. It may be that sustained ethical discussion would show that research animals are entitled to wellbeing separately from the ability of wellbeing to reduce or minimize pain and distress. However, it would not follow from any or all of these things that it is useful to include promotion of wellbeing in the *definition of refinement*. Many issues remain regarding how to understand and minimize distressful mental states.⁹ There appear to be even more as yet unresolved issues regarding how to define positive mental states in animals and whether and how to promote these states while improving or not compromising the quality of research. It may be useful to keep these 2 kinds of issues separate, and not add to the already challenging task of developing and implementing methods of refinement, defined as ways of reducing distress, potentially even more difficult conceptual, empirical, and ethical issues relating to animal wellbeing. Not including promotion of wellbeing in the *definition of refinement* need not in any way hinder the research community from considering what wellbeing is, and how, when, and why it should be provided to research animals.

Conclusion

Since the publication of *The Principles of Humane Experimental Technique*, scientists and government agencies around the world have endorsed replacement, reduction, and refinement as essential tools for promoting the humane treatment of research animals. However, as we have illustrated, there are currently in use a number of significantly different definitions of the 3Rs, and many of these definitions, although attributed to Russell and Burch, differ significantly from the definitions in the *Principles*. Some of these definitions are also unclear. As Russell and Burch emphasized, the definitions of key concepts used in any scientific endeavor express the fundamental aims and priorities of that endeavor. The definitions of replacement, reduction, and refinement in the *Principles* were crafted with an overriding and clearly expressed aim: the reduction and, whenever possible, the elimination of animal distress consistent with the conduct of sound science. Put another way, it was not the *use* of animals in research that Russell and Burch found problematic, but the infliction on research animals of *unnecessary or avoidable pain, fear, stress, anxiety, bodily discomfort and other significantly unpleasant feelings*.

Russell and Burch's original definitions of the 3Rs, we submit, have much to recommend them. Few in the scientific community disagree with the principle that when it is scientifically appropriate to use animals in research or testing, all reasonable efforts should be made to minimize and, when possible, eliminate distress experienced by these animals. In addition, in seeking to reduce or eliminate significantly unpleasant experiences in research animals, Russell and Burch's definitions of the 3Rs focus on experiences that most scientists are comfortable attributing to animals and regarding which there has been and continues to be considerable scientific study: pain, distress, fear, and various forms of bodily discomfort. In contrast, as we note earlier, not everyone would agree that animals used in research are entitled to *comfort, pleasures, wellbeing, or happiness*. Moreover, it may not be clear what these concepts *mean* if applied to animals, when research animals in fact might experience such mental states, and how promoting such mental states (assuming research animals can experience them) would affect the results and costs of research.

It is not our aim here to reject such aspects of some definitions of the 3Rs or to assert definitively that the original definitions in the *Principles* are preferable to other current or possible defini-

tions of the 3Rs. However, because the concepts of replacement, reduction, and refinement will surely remain central in animal research, it is incumbent upon the scientific community to adopt the best possible definitions of the 3Rs—definitions that reflect defensible aims and priorities and that advance both the proper treatment of animals and the quality of research and testing. We have argued that the first step in this process must be careful study of the definitions of the 3Rs in the *Principles*. Definitions of the 3Rs should be attributed to Russell and Burch only when warranted. Where definitions of replacement, reduction, or refinement reflect aims and commit scientists to approaches that differ from those endorsed in the *Principles*, these definitions should be subjected to careful questioning and consideration. It is imperative that the scientific community put all the leading current definitions of the 3Rs (including those in the *Principles*) on the table; acknowledge when there are differences in these definitions; clarify and carefully assess the advantages and disadvantages of these definitions; and choose the best features from among them. Even if this process results in modifications in or significant departures from the definitions of the 3Rs in the *Principles*, such modifications or departures will have been made knowingly and supported by explicit and persuasive argument. As a result, hopefully, all who seek to improve the treatment of research animals will employ the same definitions of replacement, reduction, and refinement—and will use definitions that express sound scientific and ethical aims. This is precisely what Russell and Burch sought to achieve.

Special Note

First published in 1959, *The Principles of Humane Experimental Technique* was republished in 1992 in a Special Edition (with a new foreword) by the Universities Federation for Animal Welfare. Although the book is currently out of print, the Johns Hopkins University Center for Animal Testing has made the Special Edition available at the following website: http://altweb.jhsph.edu/pubs/books/humane_exp/het-toc. The Internet version is not paginated and does not indicate the original pagination of the book. Page references in the current article are to the Special Edition.

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