Recommendations for standardised description of, and nomenclature concerning, oxidatively damaged nucleobases in DNA.

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Despite being a relatively young field, the study of oxidative stress has attracted huge interest. With the advent of simple, and relatively inexpensive assays (sometimes from commercial suppliers) a growing number of groups have been able to assess oxidatively generated DNA damage in mammalian cells. Whilst this is good for raising the profile of the field of oxidative stress research, it has led to an increasing number of issues when the work is written up for publication and included in grant applications. In particular, it is evident to experts in the field, editors and referees alike that there is often uncertainty concerning what is appropriate and accurate terminology, when describing studies concerning the effects of oxidatively generated DNA damage. For this reason, we wish to raise a number of points for discussion, incorporating our recommendations on this subject. The aim is to support those embarking on studies involving oxidatively generated damage to DNA nucleobases, and to produce greater uniformity across the field. We do not wish to be dogmatic, but to present a well-argued rationale for our recommendations.

**Terminology**

The term “oxidative DNA damage” has been used extensively within the literature. However, it can be misleading, as it implies that the damage i.e. the lesion *per se*, is oxidative and thus capable of oxidising other substrates. Most oxidised bases, with the exception of five hydroxyl radical-mediated thymine hydroperoxides including 5-(hydroperoxymethyl)uracil and *cis* and *trans* 5-(6)-hydroperoxy-6-(5)-hydroxy-5,6-dihydrothymine, do not exhibit oxidising properties. The same point may be made regarding the lesions themselves and also the corresponding repair enzymes, so the terms ‘oxidative lesion’, and ‘oxidative DNA glycosylase’ are similarly incorrect.
Furthermore, if we are to take ionising, or ultraviolet radiation as examples, the term used is radiation induced DNA damage, never ‘radiative’ DNA damage. Whilst we accept that the term ‘oxidative DNA damage’ is simple, widely used, and understood by the community, in the interests of accuracy, we would encourage replacing this with such terms as ‘oxidatively damaged DNA’, or ‘oxidatively generated DNA damage’, to describe the consequence of the interaction of reactive oxygen species with DNA.

**Nomenclature**

We would also like to draw attention to the nomenclature used to describe modified nucleobases, 2’-deoxyribonucleosides and ribonucleosides. Although a single letter should represent nucleobases in DNA (e.g. G, C, A, T), it is preferable to write the complete abbreviation when referring to monomers e.g. Gua for the nucleobase, dGuo for the 2’-deoxyribonucleoside, and Guo for the ribonucleoside. The oxidatively generated nucleobase which has received, by far, the most attention is 8-oxo-7,8-dihydroguanine (which should be abbreviated as 8-oxoGua; Figure 1a). This is our recommended name, based upon advice from the International Union of Pure and Applied Chemistry (IUPAC) which states that, based upon current nomenclature, the (di)hydro- prefix is non-detachable, meaning that it is always immediately before the parent name (1). On this basis, 7,8-dihydro-8-oxoguanine and 7-hydro-8-oxoguanine would be incorrect. Furthermore, 8-oxo-2’-deoxyguanine is a confusing name derived from a combination of both nucleobase and 2’-deoxyribonucleoside. For clarification, the 7,8-dihydro description is used to indicate the saturation of the double bond between N7 and C8 atoms of the parent unmodified guanine, from which the damaged nucleobase is derived (hence 8-oxo-7,8-dihydroguanine is the 8-oxo-substituted derivative of 7,8-dihydroguanine). 8-Hydroxyguanine is a frequently used term, and
indeed one used by Chemical Abstracts. However this is, in fact, a rather minor tautomer at physiological pH (2) compared to the predominant 6,8-diketo form, as inferred from NMR studies of the lesion in duplex DNA (3,4) and as the 2’-deoxyribonucleoside (5), which is further confirmed by theoretical calculations (6-8). By implication, 8-oxo, rather than 8-hydroxy, would be the form present in greatest amounts in biological systems, and hence the more accurate term to use when describing this lesion in vivo. Of course, this does not prevent the use of 8-hydroxy, if that is the specific tautomeric form in question (9). There is precedent for this proposal: the malondialdehyde-, or base propenal-derived modification of guanine, known as ‘M$_1$G’ [3-(2’-deoxy-β-D-erythro-pentofuranosyl)pyrimido[1,2-α]-purin-10(3H)-one], is a ring-closed species as a nucleobase at pH 7, but undergoes ring-opening at alkaline pH (10), and also when base-paired with dC in duplex DNA (11), when it becomes the N2-(3-oxo-1-propenyl)-dG adduct. Nonetheless, the name remains M$_1$G (or M$_1$dG, for the 2’-deoxribonucleoside equivalent), since this is the form predominating at pH 7 (10,11). Hence our proposal to define the names, based upon the major structure at pH 7, and biological context. The above advice also applies to 5,6-saturation products of thymine (Thy), cytosine (Cyt) and uracil (Ura). For example, 5,6-dihydroxy-5,6-dihydrothymine (thymine glycol; Figure 1b).

The corresponding 2’-deoxyribonucleoside of 8-oxoGua is 8-oxo-7,8-dihydro-2’-deoxyguanosine, abbreviated as 8-oxodG or 8-oxodGuo (Figure 1c). For completeness, it is worth noting that the ribonucleoside equivalent is 8-oxo-7,8-dihydroguanosine, abbreviated as 8-oxoGuo (Figure 1d).
Conclusions

An immediate benefit of achieving harmony in the terms used to describe oxidatively modified DNA, and its constituents, would be the simplification of literature searches. One keyword could be used, instead of multiple variants thereof. A further benefit, evident to authors and readers alike, would be the removal of any doubt as to what lesion is being discussed. For example, confusion between modified free nucleobase, ribonucleoside and 2’-deoxyribonucleoside hampers meaningful interpretation, leading to suggestions that modified 2’-deoxyribonucleosides, in extracellular matrices, are products of base excision repair. By the same token, confusion over the choice of abbreviation, for example, 8-oxoGua, 8-oxoG and 8-oxoGuo, can make it unclear as to whether the modified nucleobase or ribonucleoside is being described. Standardisation of nomenclature would address this problem. Therefore, whilst trivial, or common, names may continue to be used, and feature prominently in existing literature, we strongly recommend a progressive move towards consensus on the use of 8-oxo-7,8-dihydroguanine nomenclature, as indicated by IUPAC, and supported by others in the field (for example, Griffiths et al. (12)). For clarity, we recommend that assays which detect oxidatively generated DNA lesions by indirect, antibody- or enzymic-detection (e.g. comet assay), report lesions with reference to presence in DNA such as ‘8-oxoGua in DNA’.

The year 2009 marked the 25th anniversary of Kasai and Nishimura’s publication concerning 8-oxoGua in Nucleic Acids Research (13), which was closely followed by another, describing the formation of 8-oxoGua in DNA (14). It is therefore perhaps pertinent that we are considering some of the issues surrounding the nomenclature and measurement of this and related lesions. The widespread, and increasing, interest in this particular marker of oxidative stress may be explained, in part, by its apparent
omnipresence in cellular DNA *in vivo*, its biological significance, and its relatively straightforward quantification, using techniques such as HPLC-EC (15), and isotope-dilution LC-MS/MS (16). This has provided a great impetus to the study of oxidatively damaged DNA, with emphasis moving from damage *per se*, to other downstream biological events, such as repair and mutagenesis. This also explains why major efforts have been made, for example, via the European Standards Committee on Oxidatively Damaged DNA (ESCODD) (17), European Standards Committee on Urinary (DNA) Lesion Analysis (18) (ESCUA; http://escula.org) and European Comet Assay Validation Group (19-21), to resolve major discrepancies between the data provided by the available chemical and biochemical methods of measurement of 8-oxoGua/8-oxodGuo, that can vary between reports by up to $10^3$. Clearly, we have come a long way in twenty-five years, but there remains a great deal that we do not understand about this molecule, that is an ubiquitous marker of several oxidation reactions mediated by hydroxyl radical, one-electron oxidants and singlet oxygen (22).
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References


Figure legend

**Figure 1.** Structure of oxidatively generated compounds referred to in this letter.
(a) 8-oxo-7,8-dihydroguanine

(b) 5,6-dihydroxy-5,6-dihydrothymine (thymine glycol)

(c) 8-oxo-7,8-dihydro-2′-deoxyguanosine

(d) 8-oxo-7,8-dihydroguanosine

Figure 1.