

Detection of Endogenous and Exogenously Derived Ethylene Oxide DNA Adducts using ^{14}C and ^3H -Accelerator Mass Spectrometry

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Ethylene oxide (EO) is a widely used intermediate in the chemical industry and is formed in humans through metabolic oxidation of ethylene, which is generated during normal physiological processes. EO is classified as a human carcinogen and is a direct acting alkylating agent that binds primarily to the *N7*-position of guanine, forming *N7*-(2-hydroxyethyl)guanine (*N7*-HEG). In order to conduct accurate human risk assessments for occupational exposure to EO, it is necessary to ascertain the relative contribution of endogenous versus exogenously derived DNA damage. To address this issue and define the *in vivo* dose-response relationships, we have utilized a dual isotope approach, combining LC-MS/MS and HPLC-accelerator mass spectrometry (AMS) analysis for the quantification of endogenous and exogenous *N7*-HEG adducts, respectively, in tissues of exposed rats.

Administration of [^{14}C]-EO to rats (0.0001-0.1 mg/kg daily for three days) produced blood concentrations of [^{14}C]-EO equivalents of 0.1-36 pg/mg, as measured by liquid scintillation counting. This range encompasses the levels reported in blood from humans occupationally exposed to EO. AMS analysis revealed a dose-related increase in [^{14}C]-*N7*-HEG in rat spleen, stomach, and liver, with induced damage spanning from 0.002-4 adducts/ 10^8 nucleotides. Importantly, compared to the high background of *N7*-HEG naturally present (in the region of 3 adducts/ 10^8 nucleotides), the extent of exogenous damage generated was insignificant in all but one case (liver, 0.05 mg/kg). This proves that endogenous EO production is the major contributor to *N7*-HEG levels found *in vivo*. However, at the two highest doses, [^{14}C]-EO exposure caused a significant increase in endogenous *N7*-HEG formation in liver and spleen, suggesting EO can induce physiological pathways responsible for ethylene generation *in vivo* and thereby indirectly promote *N7*-HEG production. Evidence from subsequent *in vitro* studies suggests a novel mechanism of adduct formation to explain this phenomenon, involving oxidative stress and 1-aminocyclopropane-1-carboxylic acid as a potential biosynthetic precursor to ethylene in mammalian cells. Based on the proposed pathway, *N7*-HEG may have potential as a biomarker of cellular oxidative stress.

Implications: For compounds that are produced endogenously such as EO, low exogenous exposures may be overwhelmed by the background levels, resulting in no detectable significant increase in DNA damage. To assess the importance of this phenomenon to the regulation of EO exposures, it is essential to determine the variation in *N7*-HEG levels in 'unexposed' control human populations, and to identify the factors modulating this background. Similar studies on populations exposed to low levels of exogenous EO would then enable determination of whether a practical threshold exists and if a dose range could be established for this. The presence of background levels of EO must be borne in mind when interpreting past and future epidemiological studies.

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Presentations:

Marsden, D.A., Jones, D.J.L., Lamb, J.H., Tompkins, E.M., Crookston, R.J.R., Farmer, P.B., and Brown, K. (2005). Development of an LC-MS/MS method for quantifying *N7*-(2-hydroxyethyl)-guanine adducts. 35th Annual Meeting of the European Environmental Mutagen Society, Kos, Greece. Abstract published in *European Journal of Genetic and Molecular Toxicology* 155: 183.

Brown, K. (2006). Accelerator mass spectrometry for DNA adduct detection. ECNIS Sponsored Workshop on Biomarkers of Exposure and Cancer Risk: DNA Damage and DNA Adduct Detection & 6th GUM-³²P-Postlabelling Workshop, German Cancer Research Centre, Heidelberg, Germany. Abstract published in *European Journal of Genetic and Molecular Toxicology* 13: O5.

Marsden, D.A., Farmer, P.B., Jones, D.J.L., Lamb, J.H., Tompkins, E.M., Crookston, R.J.R., and Brown, K. (2006). Determination of *N*7HEG adducts in ethylene oxide treated rats using LC-MS/MS. AACR 97th Annual Meeting, Washington, DC. Abstract published in *Proceedings of the American Association for Cancer Research* V47: 703.

Marsden, D., Farmer, P.B., Jones, D.J.L., Lamb, J.H., Tompkins, E.M., Crookston, R.J.R., and Brown, K. (2006). Measurement of endogenous and exogenously derived *N*7-HEG adducts in ethylene oxide treated rats using LC-MS/MS. Joint Congress of the United Kingdom Environmental Mutagen Society and the British Toxicology Society, Warwick, UK. Abstract published in *Mutagenesis* 21(293): 55.

Marsden, D., Jones, D.J.L., Lamb, J.H., Crookston, R.J.R., Farmer, P.B., and Brown, K. (2006). Assessment of the relative contribution of exogenous and endogenously derived *N*7-(2-hydroxyethyl)guanine adducts in ethylene oxide treated rats. ECNIS Sponsored Workshop on Biomarkers of Exposure and Cancer Risk: DNA Damage and DNA Adduct Detection & 6th GUM-³²P-Postlabelling Workshop, German Cancer Research Centre, Heidelberg, Germany. Abstract published in *European Journal of Genetic and Molecular Toxicology* 31: 5.

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Marsden, D.A., Jones D.J.L., Britton, R.G., Ognibene T.J., Ubick, E., Farmer, P.B., and Brown, K. (2008). Dose response relationships for *N*7-(2-hydroxyethyl)guanine induced by low dose [¹⁴C]-ethylene oxide in rats: Evidence for a new mechanism of endogenous adduct formation. AACR 99th Annual Meeting, Washington, DC. Abstract published in *Proceedings of the American Association for Cancer Research* V49: #1886.

Brown, K. (2008). Dose response relationships for *N*7-(2-hydroxyethyl)guanine induced by low dose [¹⁴C]-ethylene oxide: Assessing the relative contribution of endogenous and exogenously derived damage. ECETOC Workshop on the Significance of DNA Adducts: Part II, Cavtat, Croatia.

Peer-reviewed publications:

Marsden, D.A., Jones, D.J.L., Lamb, J.H., Tompkins, E., Farmer, P.B., and Brown, K. (2007). Determination of endogenous and exogenously derived *N*7-(2-hydroxyethyl)guanine adducts in ethylene oxide treated rats. *Chemical Research and Toxicology* 20: 290-299.

Marsden, D.A., Jones, D.J.L., Britton, R.G., Ognibene, T., Ubick, E., Farmer, P.B., and Brown, K. (2009). Dose response relationships for *N*7-(2-hydroxyethyl)guanine induced by low dose [¹⁴C]-ethylene oxide: evidence for a novel mechanism of endogenous DNA adduct formation. *Cancer Research*. (In press).

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