Interlaboratory standardization and validation of DNA adducts postlabelling methods for human biomarker studies

Improved protocols for the phosphorus-32-postlabelling assay for the detection of carcinogen-deoxyribonucleic acid (DNA) adducts were devised and tested. The intention was to reverse the drift of different investigators using increasingly divergent experimental conditions. This would lead to a more standardized assay that can be used in future applications by different investigators for the monitoring of human exposure to genotoxic agents, permitting more meaningful comparisons among different studies or among different participants in the same study. As part of this process, there was perceived to be a need for carcinogen-modified DNA standards of known levels of adducts for use as positive controls, as standards for normalization of results with unknown samples, and to assist interlaboratory comparisons. The preparation of characterized DNA standards modified by benzo[a]pyrene (BaP, a polycyclic aromatic hydrocarbon, PAH), 4-aminobiphenyl (ABP, an aromatic amine), 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP, a heterocyclic amine) and N-methyl-N-nitrosourea (MNU, a methylating agent, to yield DNA containing 6-methylguanine) was carried out. A critical appraisal of all aspects of the phosphorus-32-postlabelling procedure and investigations to examine the influence of a number of key variations on the assay was conducted. There followed testing of a consensus protocol in a first interlaboratory trial involving 25 participants in Europe and the US, conducted on the prepared synthetic DNA standards, the assessment of interlaboratory variability and the reasons for it. The revision of the protocols and further testing in a second interlaboratory trial in which liver DNA from mice treated with BaP or ABP were assayed together with the synthetic DNA standards. A recommended set of procedures have been developed for the detection and quantification of DNA adducts formed by PAHs, aromatic amines, and methylating agents. These trials have led a much clearer idea as to what are the critical features and procedures of the phosphorus-32-postlabelling assay, and there is a set of standard DNA samples for use in quality control and against which biological samples can be normalized. These findings and adoption of recommended procedures will assist in future human studied in which it is necessary to include or compare biomonitoring from different laboratories.

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