Analytical Methods in Environmental Effects-Directed Investigations of Effluents

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Effluent discharges are released into aquatic environments as complex mixtures for which there is commonly no knowledge of components functioning as toxicants, or a lack of understanding of how toxicants interact with other effluent components. Effects-directed investigations are chemical manipulations directed by a biological endpoint and are designed to focus analytical efforts on compounds of concern in complex mixtures. Such investigations consist of extraction and iterative fractionation steps designed to isolate biological activity in successive sub-fractions until their chemical complexities are reduced to the point that candidate chemicals can be characterized. From a review of the literature, there is a commonality in the analytical approaches employed in effects-directed investigations of effluents for mutagenic and non-mutagenic substances. Most studies have employed extraction techniques to isolate and concentrate bioactive substances. Since the mid-1980s, these have evolved from the use of XAD resins to C-18 solid phase extraction (SPE). Blue cotton, blue rayon and blue chitin have been specifically used in mutagenicity investigations where PAC are involved or suspected. After isolation, subsequent fractionations have been accomplished using SPE or a high pressure liquid chromatography (HPLC) system commonly fitted with a C18 reverse phase column. Substances in active fractions are characterized by GC-MS and/or other spectrometric techniques for identification. LC-MS methods have been developed for difficult-to-analyze polar substances identified from effects-directed studies, but the potential for LC-MS to identify unknown polar compounds yet to be fully realized. *Salmonella*-based assays have been utilized to drive most fractionations in mutagenic investigations. Development of miniaturized *in vitro* bioassays, genetically or mechanistically-linked to *in vivo* effects, has allowed the pursuit of compounds associated with a range of mutagenic and non-mutagenic effects. Care in the choice of bioassay must be exercised to ensure meaningful results can be extrapolated to higher levels of biological organization. The success of effects-directed investigations should be based on a realistic initial objective of each project. Information on which compound classes are involved is frequently achieved; confirmation of chemical-specific causes is much more difficult and should not be used as a criterion of success.

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