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## Carcinogenesis Assay of Subfractions of Cigarette Smoke Condensate Prepared by Solvent-Solvent Separation of the Neutral Fraction<sup>1,2</sup>

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**SUMMARY**—Carcinogenesis assay was conducted on subfractions of the neutral fraction (NF) of cigarette smoke condensate, subfractions that were prepared by solvent partition. Of the 2 major subfractions, the "methanol-insoluble" neutrals (MIN) were much more active than the "methanol-soluble" neutrals (MSN). Distribution of MSN between nitromethane and carbon disulfide yielded 2 active fractions. Poor dose-response effects suggest that extraneous materials may reduce the activity of MSN. Analysis of the recovery of MSN is difficult, but apparently there were significant losses during separation. On countercurrent distribution (CCD), MIN yielded 3 active and 2 inactive subfractions. Recovery of the activity of MIN in the subfractions was excellent in comparison with an earlier separation with silicic acid chromatography. Recoveries might be improved if CCD is applied to the NF before the more rigorous chromatographic separations.—*J Natl Cancer Inst* 49: 477-483, 1972.

IN AN earlier study, 4 of the subfractions prepared by silicic acid chromatography of the neutral fraction (NF) of cigarette smoke condensate (CSC) induced skin tumors in mice previously painted with 125  $\mu$ g of 7,12-dimethylbenz[a]anthracene (DMBA) (1). With this procedure, tumor-promoting agents as well as complete carcinogens were detected. All the subfractions were recombined in proportion to their yield, to provide a reconstituted sample that would have been identical with NF if no changes had occurred during the separation procedure. The biological activity of this reconstituted material, however, was substantially less than that of NF, suggesting that important amounts of the active materials were lost during chromatography. The current study was undertaken to examine solvent partitioning alone as means of

fractionating NF, with the hope that these losses could be avoided.

## MATERIALS AND METHODS

Details of the preparation of the various fractions will be described elsewhere (2). Briefly, 1-kg

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