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Predictive Model for Determining Asbestos Concentrations for Fibers Less Than Five Micrometers in Length

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The controversy of whether small asbestos fibers are biologically significant has not been resolved. The present standard method for evaluating asbestos fiber concentrations in workroom air excludes fibers less than 5 μm long even though it has been shown that small fiber concentrations dominate in a dust cloud. This research project was conducted to develop a mathematical model whereby one could predict small (<5 μm length) asbestos fiber concentration based on the fiber count concentration determined by phase contrast microscope analysis. Dry chrysotile asbestos was aerosolized into a chamber and sampled by membrane filtration. Segments from each filter were analyzed by both the NIOSH technique using phase contrast microscopy (PCM) and scanning electron microscopy (SEM) at 2000× for fiber concentrations. A linear relationship was found to exist between the natural logarithm of the SEM-determined concentration and the natural logarithm of the PCM-determined concentration ($r = 0.852$). Using these data, a mathematical model was developed to predict SEM concentrations based on PCM counts. This model may have application in retrospective epidemiological studies for estimating small fiber exposure levels to determine if small fibers play a role in disease production. The greatest utility would be in those retrospective studies where the only exposure information available is based on PCM counts. © 1987 Academic Press, Inc.

INTRODUCTION

The adverse health effects related to exposure to asbestos have been well documented (Selikoff and Lee, 1978). With the recognition of asbestos as a disease-causing agent, many researchers have undertaken the task of determining what properties of asbestos are responsible for disease causation and what amount is hazardous. Unfortunately, little light has been shed on the issue. It is not known what attributes of asbestos constitute a health hazard, nor is it known what degree or period of exposure is truly hazardous (Selikoff and Lee, 1978; Levine, 1985; Doull *et al.* 1975). One area of major controversy is whether or not short (<5 μm) asbestos fibers are significant in disease production.

The idea that some critical fiber length may be important in asbestos-related diseases first arose in 1946 (King *et al.*, 1946). Chrysotile fibers were administered to rabbits by intratracheal injection. A greater tissue reaction was reported in those animals that received the long-fiber doses. A study in 1951 indicated similar results in that animals which had inhaled chrysotile fibers in the range of 20–50 μm had more lung fibrosis than those breathing only fibers less than 3 μm (Vorwald *et al.*, 1951). Other studies followed which also indicated that the longer fibers had a greater disease-producing potential (Timbrell and Skidmore, 1968; Stanton and Wrench, 1972).

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Conversely, there has been research which indicates that the opposite is true. One study found that short asbestos fibers injected intrapleurally produced more mesotheliomas than long fibers (Wagner *et al.*, 1973). In addition, crocidolite mined in the northern Cape Province of South Africa is frequently associated with pleural mesothelioma whereas fewer cases have been reported from the South African Transvaal, which has thicker and longer fibers (Levine, 1978). In research comparing the biological effects of pure asbestos-free talc with superfine chrysotile asbestos by intrapleural injection in rats, it was reported that 18 rats in the chrysotile group developed mesothelioma while no tumors were seen in those given talc (Wagner *et al.*, 1975). Another study found that a chrysotile sample with 99.8% of the fibers less than 5 μm in length produced mesotheliomas in 32% of the animals tested (Pott *et al.*, 1976).

Although shorter fibers are preferentially cleared from human lungs, autopsy studies of asbestos disease victims have consistently shown that the fibers shorter than 5 μm in length are by far the predominant fiber found in human lungs (Rendall and Skikne, 1980; Davis and Conlan, 1973; Fondimore, 1975; Langer *et al.*, 1971, 1979; Timbrell, 1979). This indicated that fewer long fibers were reaching the lower pulmonary spaces.

There are extensive data indicating that both short (<5 μm) and long fibers are biologically active (Rendall and Skikne, 1980; Langer *et al.*, 1971; Davis *et al.*, 1978). Short asbestos fibers in the alveolar region are engulfed by pulmonary macrophages (Langer *et al.*, 1979; Lemen and Dement, 1979; Morgan, 1979). These phagocytosed short fibers gain entrance into one of the target cells which may induce carcinoma. It has been pointed out that if fiber length is indeed important, then crocidolite, which produces the shortest fiber, should possess the least disease potential (Davis *et al.*, 1978; Langer *et al.*, 1974). Yet conversely, crocidolite produces far more mesotheliomas than other asbestos types in addition to a marked fibrosis. It is obvious that fiber length cannot be the only factor in producing disease. Selikoff (Selikoff and Lee, 1978) succinctly sums up the controversy by stating: "The only safe conclusion concerning the relative activities of short and long asbestos fibers is that there is no firm conclusion from the present evidence."

Asbestos exposure standards discriminate against fibers below the limit of good light microscope resolution (less than 5 μm with the present standard). This practice has continued even though it has been estimated that for every fiber greater than 5 μm long there may be as many as 100 fibers too short or thin to be visible (Selikoff and Lee, 1978; Langer *et al.*, 1974; ACGIH, 1980).

In order to determine small fiber concentrations electron microscope techniques are necessary. For practical reasons, however, it is not possible to evaluate every sample by electron microscopy. Thus a statistically valid method for predicting small (<5 μm length) asbestos fiber concentration by use of the concentration determined by phase contrast microscopy (PCM) would be useful in reconstructing the past expposure experience of workers. A model such as this is necessary if the controversy regarding the relative health effects of short and long fibers is ever to be resolved through epidemiology. Such a model would greatly enhance attempts to determine the role of small fibers in pathogenesis in retro-

ective epidemiological studies where air concentrations reported were only for fibers greater than 5 μm in length. The objective of this research project was to develop a predictive model for determining asbestos fiber concentrations for fibers less than 5 μm in length based on PCM-analyzed air samples.

MATERIALS AND METHODS

Ratios between electron microscope and optical microscope fiber counts have been shown to vary according to the workplace, general environment, fiber type, and process (Levine, 1978). In order to minimize such variability, chrysotile (99% pure) was chosen as the test asbestos. Additionally, it is the most commonly encountered asbestos type in the United States.

Dry chrysotile was weighed and placed in Misto$_2$ Gen aerosol generator. Compressed air (100 psig) was used to disperse the dry sample in a 1.0-m^3, lined, Plexiglas chamber. Nuclepore filters (37 mm, 0.4-μm pore size in three-piece face cassettes) were used for sampling the chrysotile. Trial runs were conducted with two objectives: (a) to determine the mass of chrysotile necessary to obtain a chamber concentration in the TLV region (2 fibers/cc) and (b) to determine the optimum sampling time for achieving a fiber deposition of 1 to 5 fibers per microscope counting field. The sampling parameters found to meet these objectives are listed in Table 1.

A total of 32 airborne asbestos samples were collected. Eight sample runs were made in which four air samples were collected during each run. Two air samples were lost from filters dropping on the floor or tabletop when being removed from cassettes. This left a total of 30 samples for analysis. After collection all samples were coded by an independent observer so that the analyst was unaware of sample identification and sampling conditions. A section from each filter was analyzed by both PCM and scanning electron microscopy (SEM). All samples were first analyzed by PCM, returned to the independent observer for recoding, and then analyzed by SEM.

All filters were analyzed by PCM (400×) according to NIOSH fiber definitions and counting rules (NIOSH, 1979). Chloroform was used for the mounting media, and a porton reticle was used to delineate counting fields and to measure fiber length. As a quality control measure recounts were made on ten filters. A comparison of the recounts to the original fiber counts showed good analytical correlation. Additionally, nine filters were analyzed for size-count distributions by the truncated multiple traverse method (TMT) (Buchan, 1972). Each traverse

TABLE 1
SAMPLING PARAMETERS USED FOR DATA COLLECTION

Sample mass (mg)	Sampling time (min)	Flow rate (liters/min)
0.5	180	1.0
1.0	60	1.0
2.0	30	1.0

consisted of ten fields and an end point of twenty fibers per porton reticle circle size was sought.

For SEM analysis, a 3 × 5-mm section of each filter was mounted on a strip of aluminum tape on a 14-mm aluminum stub. A sputter coater was used to coat the filter samples with approximately 300 Å of gold palladium. After coating, the specimens were kept under vacuum until analysis. A Hitachi Perkins–Elmer Hi-Scan Model HHS-2R scanning electron microscope equipped with a secondary electron detector was used for SEM fiber analysis. An accelerating voltage of 25 keV, a working distance of 15 mm, and a magnification of 2000× provided sufficient resolution for submicron fiber determination and a counting field area large enough to obtain statistically useful results.

The fibers were sized and counted directly from the CRT screen of the SEM. The NIOSH counting method was utilized with the exception that all fibers (both greater than and less than 5 μm long) were counted. Each filter analyzed by PCM for size distributions using the previously described TMT method was similarly analyzed by SEM. Fibers were classified into groups by use of a ruler corresponding to the porton reticle circle diameters.

For each analysis, the asbestos concentration in fibers per cubic centimeter (fibers/cc) was entered into a computer file for statistical analysis. The mean asbestos concentrations from the SEM and PCM methods were compared statistically via the paired t test applied to the logarithms of the respective concentrations. Regression analysis of the SEM asbestos concentration versus the PCM concentration (both in logarithms) was utilized to develop the predictive model. For the nine pairs of data analyzed for size distributions, the total fibers for each circle size were computerized and used to determine, for both methods, the geometric mean and geometric standard deviation of the distribution of asbestos lengths, as well as to statistically evaluate the fitted log-normal distribution. Stepwise regression analysis (MINITAB, Release 81.1) was utilized to determine if the size distribution data from the truncated multiple traverse analysis (i.e., geometric mean, geometric standard deviation, and size percentile) would significantly improve the predictive model.

RESULTS

In this research, the PCM-derived fiber concentrations are significantly lower than the SEM fiber concentrations ($P < 0.001$). Table 2 gives the range, mean, and standard deviation of the fiber concentrations obtained by each method for the three quantities of asbestos generated. The average SEM to PCM fiber concentration ratio was 2.98.

All of the size distributions obtained by PCM and SEM analysis were log-normal, with correlation coefficients (r) greater than .970 ($P < 0.001$). The geometric mean fiber length for the PCM method was significantly greater than that for the SEM analysis ($P < 0.001$), with a mean PCM geometric mean of 3.87 μm and a mean SEM geometric mean of 0.75 μm. The difference in the mean geometric standard deviation between the PCM method and the SEM method was highly significant (1.83 and 3.51, respectively; $P < 0.001$). Because of the large

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TABLE 2

RANGE, MEAN, AND STANDARD DEVIATION OF FIBER CONCENTRATIONS OBTAINED BY ANALYSIS METHOD AND MASS OF ASBESTOS GENERATED

Mass generated (mg)	Concentration (fibers/cc)	
	PCM	SEM
0.5	Range = 0.41–1.09 $\bar{x}$ = 0.69 s = 0.22	Range = 1.35–2.82 $\bar{x}$ = 2,18 s = 0.50
1.0	Range = 1.02–6.33 $\bar{x}$ = 2.70 s = 1.40	Range = 2.99–10.92 $\bar{x}$ = 7.29 s = 2.23
2.0	Range = 1.91–7.92 $\bar{x}$ = 4.00 s = 1.10	Range = 7.96–19.18 $\bar{x}$ = 11.35 s = 3.87

number of small fibers counted with the SEM method, these results were not unexpected.

A strong correlation ($r = .852$, $P < 0.01$) was obtained between the natural logarithm of the SEM fiber concentration and the natural logarithm of the PCM fiber concentration. The results from the stepwise regression analysis indicated that the natural logarithm of the PCM concentration was the only significant contributor to the regression equation. This may have been due to the limited amount of truncated multiple traversing data available, as well as the narrow range of these data. The regression equation obtained was

$$\ln \text{SEM concentration} = 1.25 + 0.703\ (\ln \text{PCM concentration}),$$

where the estimated y intercept was 1.25 with standard error 0.10 and the estimated slope of the line was 0.703 with standard error 0.082. The estimated standard error of the regression line was 0.378. From this regression equation, using the rules of logarithms, the following predictive model was derived:

$$\text{SEM concentration} = 3.49 \times (\text{PCM concentration})^{0.703}.$$

The predicted SEM concentrations based on this model are depicted in Table 3. The 90% confidence band for the regression line and the 90% confidence band for predicted SEM concentrations based on future PCM values were then developed. Figure 1 depicts a scatterplot of the data, the estimated regression line, the 90% confidence band for the regression line, and the 90% prediction band for estimated log-SEM concentrations based on the test PCM values. Predicted SEM concentrations, along with 90% prediction limits, for future PCM values are presented in Table 4. Although the prediction range is wide, it is evident that a strong linear relationship exists between the two log-concentrations. Also, these results emphasize the fact that the present method for evaluating asbestos fibers by PCM is at best, a rough estimate of the true concentration.

CONCLUSIONS

Because fiber size will vary depending on the fiber type and process, any work-

TABLE 3
PREDICTED SEM (SEM) CONCENTRATIONS (FIBERS/cc) BASED ON LINEAR REGRESSION ANALYSIS

Filter number	PCM concentration	Actual SEM concentration	SEM
1	3.14	8.67	7.79
2	2.61	9.48	6.85
3	3.03	5.50	7.60
4	4.03	15.72	9.29
5	7.82	11.52	14.80
6	5.61	11.78	11.72
7	4.23	19.18	9.61
8	0.62	2.60	2.49
9	1.09	2.45	3.71
10	0.73	2.82	2.79
11	0.70	1.35	2.71
12	7.92	8.16	14.94
13	2.50	8.29	6.64
14	1.91	9.36	5.50
15	4.98	7.96	10.78
16	0.41	1.80	1.86
17	0.77	2.24	2.90
18	1.63	6.07	4.92
19	3.99	6.67	9.22
20	2.94	6.61	7.44
21	1.02	2.99	3.54
22	1.67	6.34	5.00
23	6.33	7.52	12.76
24	2.38	10.92	6.42
25	1.93	9.44	5.34
26	6.29	11.68	12.70
27	3.77	8.47	8.86
28	4.50	16.50	10.04
29	4.54	7.59	12.10
30	0.48	2.02	2.08

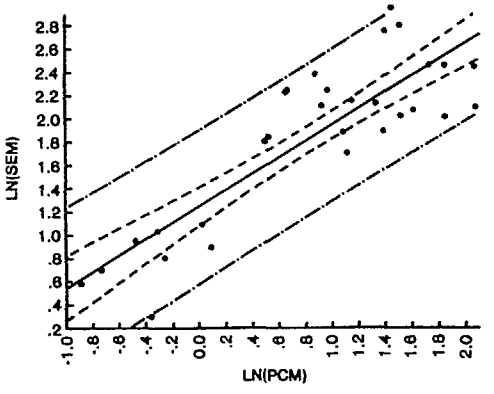
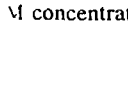


FIG. 1. Scatterplot, estimated regression line (solid line), 90% confidence band (dashed line), and 90% prediction band (dash-dot line) for natural logarithm of SEM concentrations based on the logarithms of PCM values.

D ON LINEAR REGRESSION ANALYSIS

Actual SEM concentration	SEM
8.67	7.79
9.48	6.85
5.50	7.60
15.72	9.29
11.52	14.80
11.78	11.72
19.18	9.61
2.60	2.49
2.45	3.71
2.82	2.79
1.35	2.71
8.16	14.94
8.29	6.64
9.36	5.50
7.96	10.78
1.80	1.86
2.24	2.90
6.07	4.92
6.67	9.22
6.61	7.44
2.99	3.54
6.34	5.00
7.52	12.76
10.92	6.42
9.44	5.34
11.68	12.70
8.47	8.86
16.50	10.04
7.59	12.10
2.02	2.08





confidence band (dashed line), and
M concentrations based on the loga-

TABLE 4
PREDICTED SEM FIBER CONCENTRATIONS (FIBERS/cc) AND 90% PREDICTION INTERVAL FOR HYPOTHESIZED PCM VALUES

PCM concentration	Predicted SEM concentration	90% Prediction interval
0.1	0.69	0.40–1.21
0.2	1.13	0.66–1.93
0.3	1.24	0.88–2.53
0.4	1.84	1.10–3.08
0.5	2.14	1.28–3.59
0.6	2.44	1.46–4.07
0.7	2.71	1.63–4.52
0.8	2.98	1.79–4.96
0.9	3.24	1.95–5.38
1.0	3.49	2.10–5.78
1.5	4.64	2.80–7.67
2.0	5.68	3.44–9.38
2.5	6.64	4.02–10.97
3.0	7.55	4.57–12.47
3.5	8.41	5.09–13.91
4.0	9.24	5.59–15.29
4.5	10.04	6.06–16.62
5.0	10.81	6.52–17.92
5.5	11.56	6.97–19.18
6.0	12.29	7.40–20.41
6.5	13.00	7.82–21.61
7.0	13.70	8.23–22.79

predictive model will only be valid for a particular asbestos type and process. This research demonstrated that there is a linear relationship between the natural logarithm of the SEM (actual) fiber concentration and the natural logarithm of the PCM fiber concentration for 99% pure chrysotile asbestos in a laboratory situation.

It must be reemphasized that fiber size-count distributions and concentrations will vary according to industrial and construction processes and operations. The model developed was under ideal laboratory conditions. Thus, the model described must be refined through field validation studies before a truly dependable model is available to estimate small-fiber concentrations. Nevertheless, the model presented is at least a first step and a rough tool for use in estimating past small-fiber exposures from existing PCM data. Reconstructing past exposure experience of workers to small fibers for use in retrospective epidemiological studies may assist in determining whether or not small fibers contribute to the pathogenesis of asbestos-induced disease.

REFERENCES

American Conference of Governmental Industrial Hygienists (1980). "Documentation of Threshold Limit Values," 4th ed. ACGIH, Cincinnati.

Bohan, R. (1972). "Atmospheric Dispersion of Particulate Emissions from an Activated Sludge Unit," pp. 24–26. Doctoral dissertation, Graduate College, University of Oklahoma, Norman.

Davis, J., and Conlan, S. (1973). Experimental studies on the effects of heated chrysotile asbesto and automobile brake lining dust injected in the body cavities of mice. *Exp. Mol. Pathol.* 19, 339–359.

Davis, J., Beckett, S., Bolton, R., Collings, P., and Middleton, A. (1978). Mass and number of fibers in the pathogenesis of asbestos related lung disease in rats. *Brit. J. Cancer* 37, 673–688.

Doull, J., Klaassen, C., and Amdur, M., Eds. (1975). "Casarett and Doull's Toxicology, the Basic Science of Poisons," 2nd ed., pp. 269–271. Macmillan Co., New York.

Fondimore, A. (1975). Quantitative study of the deposition of asbestos in the lung and pleura of subjects with diverse exposures. *In* "Proceedings, Symposium on the Pathology of Asbestos, Rouen, France, October 28, 1975."

King, E., Clegg, J., and Rae, V. (1946). Effects of asbestos, and of asbestos and aluminum on the lung of rabbits. *Thorax* 1, 188–197.

Langer, A., Selikoff, I., and Sastre A. (1971). Chrysotile asbestos in the lungs of persons in New York City. *Arch. Environ. Health.* 22, 348–361.

Langer, A., Mackler, A., and Pooley, F. (1974). Electron microscopical investigations of asbestos fibers. *Environ. Health Perspect.* 9, 63–80.

Langer, A., Rohl, A., Wolff, M., and Selikoff, I. (1979). Asbestos, fibrous minerals and biological properties. *In* "Dusts and Disease," pp. 87–96. Pathotox Publishers, IL.

Lemen, R., and Dement, J., Eds. (1979). "Dusts and Disease," pp. 1–13. Pathotox Publishers, IL.

Levine, R., Ed. (1978). "Asbestos: An Information Resource." DHEW (NIH) 79-181. National Cancer Institute, Bethesda, MD.

Morgan, A. (1979). Fiber dimensions: Their significance in the deposition and clearance of inhaled fibrous dusts. *In* "Dusts and Disease," pp. 87–96. Pathotox Publishers, IL.

NIOSH (1979). "USPHS/NIOSH Membrane Filter Method for Evaluating Airborne Asbestos Fibers." DHEW (NIOSH) Publication 72-127.

Pott, F., Friedrichs, K., and Huth, F. (1976). Results of the effect of fibrous dusts and their interpretation with regard to the carcinogenesis in humans. *Zbl. Bakt. Hyg. I. Abt. Orig.* 162, 467–505.

Rendall, R., and Skikne, M. (1980). Submicroscopic fibers in industrial atmospheres. *In* "Biological Effects of Mineral Fibers," Vol. 1, pp. 837–841. IARC Publication 30.

Selikoff, I., and Lee, D. (1978). "Asbestos and Disease," pp. 1–50. Academic Press, New York.

Stanton, M., and Wrench, C. (1972). Mechanisms of mesothelioma induction with asbestos and fibrous glass. *J. Natl. Canad. Inst.* 48, 797–822.

Timbrell, U., and Skidmore, J. (1968). Significance of fibre length in experimental asbestosis. *In* "Biologische Wirkungen des Asbestos Internationale Kouferenz, Dresden, Deutsches Zentralinstitut fur Arbeitsmedizin," pp. 52–56.

Timbrell, V. (1979). Measurement of fibres in human lung tissue. *In* "Symposium on Biological Effects of Mineral Fibres, IARC, Lyon, France, September 1979."

Vorwald, A., Durkan, T., and Pratt, P. (1951). Experimental studies of asbestosis. *A.M.A. Arch. Ind. Hyg. Occup. Med.* 3, 1–43.

Wagner, J., Berry, B., and Timbrell, U. (1973). Mesotheliomata in rats after inoculation with asbestos and other materials. *Brit. J. Cancer* 28, 173–185.

Wagner, J., Berry, G., Cooke, T., Hill, R., Pooley, F., and Skidmore, J. (1975). Animal experiments with talc. *In* "Fourth International Symposium on Inhaled Particles and Vapors, Edinburgh, September 1975."

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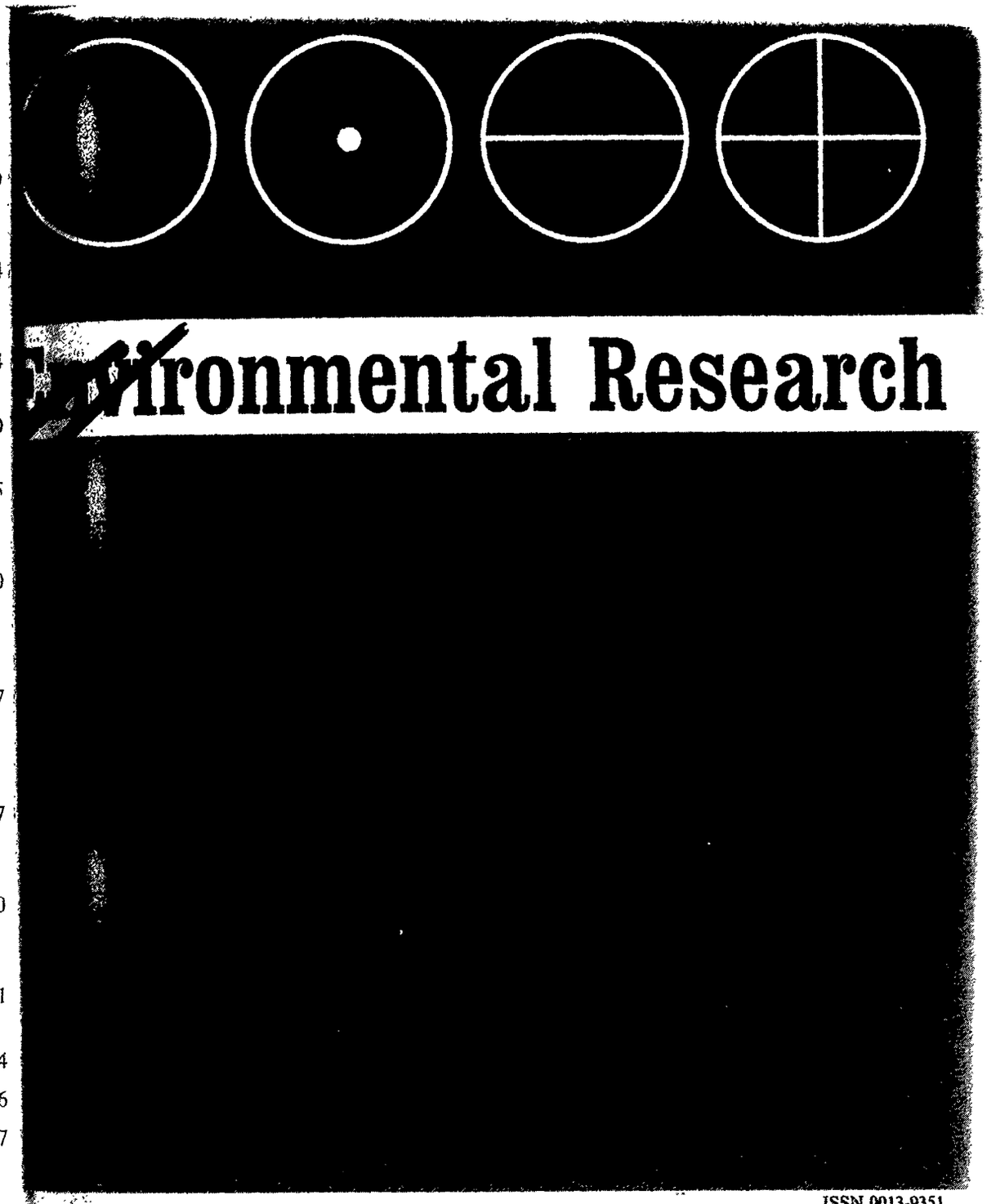
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