to inhibitors of communication in the same manner; and inhibition of intercellular communication is cell type- and density-dependent.

In conclusion, we believe that it is premature to make a general conclusion suggesting that scrape-loading is the most sensitive and/or most discriminating method for determining the effects of chemicals on GJIC.

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Reply

To the Editor:

McKarns, Bombick, and Doolittle have expressed some concerns about our paper, *Comparison of Assays for Gap Junctional Communication Using Human Embryocarcinoma Cells Exposed to Dieldrin* (Loch-Caruso *et al.,* 1990), which deserve comment.

Most importantly, we did not intend to suggest that the scrape-loading assay was the "best" assay for determination of inhibition of gap junctional communication—under all conditions, for all cells, and with all chemicals. In fact, the major focus of the work was to compare several procedures previously described in the literature with a new procedure that was developed in our laboratory. In our comparison, experimental conditions were unavoidably different for the assays, as discussed in our paper and also as pointed out by McKarns and her associates. Rather than ignore these differences, we discussed the potential contribution of the more prominent factors to the results.

McKarns *et al.* raise objections to the use of a razor blade in forming the scrape line in the scrape-loading assay. In the original description of the scrape-loading assay, El-Fouly *et al.* (1987) used a blunt probe to form an aisle by scraping across a monolayer. This in-

troduced irregularity in the scrape line that complicated quantification. In subsequent reports, El-Fouly and others have modified the procedure as we had, using a razor blade, surgical blade, or other sharp instrument, with no apparent alteration of dye transfer compared to that with the use of a blunt scraper (Blennerhassett *et al.*, 1989; De Feijter *et al.*, 1990; Madhukar *et al.*, 1989; Nicolson *et al.*, 1988; Pepper *et al.*, 1989; Suter *et al.*, 1987; Ye *et al.*, 1990).

In Fig. 2, not all of the cells along the scrape line exhibit Lucifer yellow fluorescence, suggesting that dye uptake and/or retention was not uniform along the scrape line. Numerous examples of similar variability in dye labeling along the scrape line can be found in the literature, suggesting that it is a characteristic of the assay (e.g., Dotto *et al.*, 1989; El-Fouly *et al.*, 1987; Eldridge *et al.*, 1989; Flodstrom *et al.*, 1988; Jongen *et al.*, 1987; Larsen and Haudenschild, 1988; Larsen *et al.*, 1990; Nicolson *et al.*, 1988; Pepper *et al.*, 1989; Sharovskaja *et al.*, 1988). This variability may be due to multiple factors, but should not invalidate the assay. Variability is inherent in each of the assays; as long as it is consistent across treatments, statistical analysis should resolve any treatment effects. We have verified that variability in dye uptake/retention was independent of dieldrin treatment by scoring the number of fluorescent cells scrape-loaded with the higher molecular weight, nonjunctionally transferable rhodamine dextran (unpublished results).

It is unclear to us why McKarns *et al.* find "it curious that a no-observed-effect-level was not observed with the scrape-loading assay." We predict that lower concentrations would, in fact, demonstrate this effect. We are not surprised that different procedures which measure different end-points of the same phenomenon also show differences in dose response. Possible explanations for no observable effect at the lower dieldrin concentrations, which were observed with other assays, are discussed in our paper.

The variability observed with the fluorescence return after photobleaching (FRAP) assay was of concern to us, also, and discussed in our paper. McKarns *et al.* state that this "has not been reported by other investigators," citing Wade *et al.* (1986). However, Wade *et al.* (1986) did not report quantitative fluorescence data for unbleached cells, nor did their photos include any isolated unbleached cells. In fact, their photos suggest that uncontrolled bleaching may have occurred, since reduced fluorescence is apparent in unbleached cells distant from the targeted photobleached cells (Wade *et al.*, 1986). We have identified three reports in the literature that, like ours, included unbleached cells as a control for background photobleaching (De Feijter *et al.*, 1990; Hasler *et al.*, 1990; Ye *et al.*, 1990). In the photo examples from these reports, De Feijter *et al.* (1990) and Ye *et al.* (1990) show uncontrolled bleaching in the range of approximately 3 to 12%, while the examples in Hasler *et al.* (1990) show about 35 to 40% uncontrolled bleaching. Since summary quantitative data on unbleached controls were not provided, we cannot determine whether these examples are representative of all data sets, nor can we ascertain the degree of variability of the uncontrolled bleaching in these studies. With an image analysis system, software parameters allow the opportunity to control certain aspects the images. We chose to optimize our parameters at the outset of the experiment for background subtraction, fluorescence detection sensitivity, and bleaching strength of the laser; we then maintained these parameters unchanged for all observations. Despite our efforts to optimize software parameters, we observed considerable field-to-field variability in the uncontrolled bleaching.

Finally, we look forward to the contribution of McKarns *et al.* to the published literature on their comparative findings of the scrape-loading and FRAP assays. We have found few *quantitative* comparisons of the assays. We would be pleased to learn of technical improvements that simplify and optimize FRAP measurement of gap junctional communication compared to our experiments, since, like

each of the assays, FRAP has potential advantages as well as limitations.

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To the Editor:

As the study director on all of the studies described in the recent paper by McKee *et al.* (1990, **15,** 320–328) entitled *Estimation of Epidermal Carcinogenic Potency,* I read the paper with great interest. Although I generally agree with the conclusions presented in the paper, there is one very important point that, I believe, deserves comment and clarification.

In the third section of the Results, the authors presented an "evaluation of the repeatability of dermal carcinogenesis data." They compared the slopes of the dose–response