an amplitude of less than 25 microvolts (root mean square). The effects of a proprioceptive stimulus from this response would, presumably, overlap the tone in time. The session was terminated when 500 reinforcements had been administered. For this situation there were, of course, no "False Alarms" or "Misses." The procedure is shown as the first paradigm in Fig. 2.

In sessions 3 to 6 we did not present the tone at once after observing the thumb twitch, but watched a second trace on the dual-beam oscilloscope for a deflection of the same type which represented the sub key press. If this occurred within 2 seconds, a "Hit" was recorded and the tone was presented immediately. If it did not occur, the tone was presented at the end of 2 seconds and a "Miss" was recorded.

Although, as stated earlier, the overall rates of the two responses remained much the same throughout the experiment, under paradigm 2 there was a striking increase in the number of "Hits," with a corresponding decrease in the frequency of "False Alarms" and "Misses." Most of this change is shown in the curves for the third session, with only slight additional effects obtained in three further sessions under this procedure.

Session 7 began under paradigm 2, except that each time the thumb twitch and sub key press occurred the tone was reduced in intensity, so that after 20 presentations it was completely gone, thus effecting a transition to paradigm 3. As the tone became faint, the subject complained over the intercom that it was "getting hard to hear," but was told simply to continue to respond to those tones that he did hear. In this session the cumulative record for "Hits" is for the thumb twitch followed within 2 seconds by the *overt* key press and not, as in all other sessions, by the sub key press.

Our purpose in fading out the tone was, of course, to remove the exteroceptive component from the original compound stimulus for key pressing, and thus to ascertain whether proprioceptive feedback alone could now evoke the overt response. The cumulative records under this condition are scarcely distinguishable from those for preceding days when the tone was present. The reduction in the rate of "Hits" which occurs about half way through the session and the corresponding rise in the rate for "Misses" were not present in the records for the other two subjects who progressed to this stage.

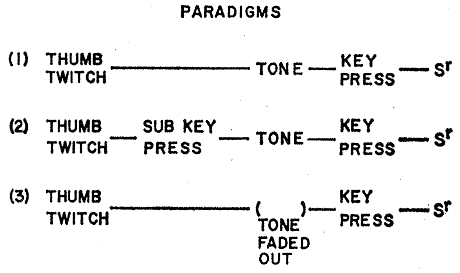

Fig. 2. Paradigms employed at various stages of experiment. $S^r$, reinforcing stimulus.

Following this session in which the tone was not presented, paradigm 2 was reinstituted for two more sessions, and the experiment was then concluded with two sessions of extinction, where neither tone nor reinforcement was presented. The extinction curves indicate an incomplete return to the original operant level rates for the three types of event. Further extinction would have been conducted, but the subject was not willing to continue.

It remains to account for the subject's continued key pressing to the tone after this was no longer exteroceptively presented. His report, when he was questioned at the end of the experiment, was that he "still heard it." This is in keeping with the literature on experimentally produced "images," "conditioned sensations," and "hallucinations" (*2*). Using a procedure very similar to ours, Stevens recently conditioned a patient to press a key when paroxysmal epileptiform activity occurred in his electroencephalogram (EEG) (*3*). She first allowed the patient to hear the abnormal activity by means of an "audio EEG" produced by feeding one channel through a speaker. When the audio signal was later removed, he continued to press the key and reported that for the first time in his life he now occasionally experienced a warning sensation prior to his petit mal attacks. Whether this sensation was of an auditory nature Stevens does not specify. (Since abnormal activity in the EEG lasting 5 seconds or more was accompanied in this subject by eye opening and rhythmic blinking, it was perhaps the onset of these discriminable movements, rather than the change in the EEG per se, which served as the basis of the sensation.)

When paradigm 2 was resumed in sessions 8 and 9, our subject responded to his thumb twitch with a key press which came faster than the experimenter's reaction time in noting the oscilloscope deflection and presenting the exteroceptive tone. The subject claimed that he sometimes heard the tone twice in rapid succession. Apparently the two kinds of discriminative stimulus, "subjective tone" and "objective tone," were not appreciably different.

Under the extinction procedure, although "Hits" continued at a high rate, the subject overtly pressed the key (and later reported that he had "actually" heard the tone) not more than four or five times during each of the 1-hour sessions. Whether the initiation of reinforcement for sub key presses would at this stage reinstate "hearing the tone" has not been determined (*4*).

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## Indophenol Blue as a Chromogenic Agent for Identification of Halogenated Aromatic Hydrocarbons

Abstract. *The metachromatic dye indophenol blue* [N-(p-*dimethylaminophenyl*)-*1,4-naphthoquinoneimine*] *is adaptable for qualitative and quantitative measurements of halogenated aromatic hydrocarbons in paper chromatographic procedures and spot tests.*

The chromogenic procedures used to define chlorinated aromatic pesticides on paper chromatograms are tedious and often noxious. Silver precipitation techniques (*1*), or the reduction of ferric sulfate (2) have been employed. Neither technique produces chromospectric differentiations of isomers or analogs superimposed on similar $R_F$ loci.

A search was made of the quinoneimine dyes for a more suitable chromogene, since these agents often form

Table 1. Approximations on sensitivities of some chlorinated aromatic pesticides to indophenol blue in micrograms per microliter, based on technical grade materials and five microliter samples.

| Pesticide | Sensitivity approximation (μg/μl) |
|---|---|
| Aldrin | 0.5 |
| Botran | 2.5 |
| Chlordane | 1.0 |
| DDD | 0.3 |
| DDT | 0.1 |
| Dichloronaphthalene | 0.5 |
| Dieldrin | 3.0 |
| Dimite | 2.0 |
| Endrin | 1.5 |
| Heptachlor | 0.7 |
| Hexachlorobenzene | 10.0 |
| Hexachlorocyclohexane | 2.0 |
| Methoxychlor | 0.5 |
| Ovex | 1.5 |
| Paradichlorobenzene | 3.0 |
| Pentachlorobenzene | 6.0 |
| Pentachloroethylbenzene | 0.3 |
| Pentachloronitrobenzene | 4.0 |
| Pentachlorophenol | 0.7 |
| Phygon | 8.0 |
| Spergon | 6.0 |
| Tedion | 0.2 |
| Tetrachloroanisole | 3.5 |
| Tetrachloronitrobenzene | 5.0 |
| Tetrachloronitrophenyl acetate | 2.5 |
| Tetrachlorophenol | 1.5 |
| Toxaphene | 2.5 |

stable complexes or are adsorbed intensely to specific chemical structures indigenous to given sorts of compounds. These dyes are notably metachromatic and sensitive to *p*H manipulations. Unfixed dyes are easily altered chromogenically and eluted from nonspecific structures with solvents of a suitable *p*H. An indophenol chromophore was found that is fixed uniquely by halogen configurations on aromatic hydrocarbons. This chromophore is indophenol blue (C.I. No. 821 or Shultz No. 939). Its chemical structure is *N*-(*p*-dimethylaminophenyl)-1,4-naphthoquinoneimine. It has an absorption maximum in alcohol of approximately 592 mμ and a molecular weight of 276.324 (*3*). In spot tests and on paper chromatograms, the dye in concentrations of 0.05 to 0.1 percent has been sensitive to those halogenated aromatics tested. It has been insensitive to all nonhalogenated aromatics tested.

To bring about the chromogenic reaction, the dye must be used in combination with one of several weak organic acids. Those found most suitable were formic, lactic, and citric acids. For some reason acetic acid did not react suitably. Arbitrarily, 20 percent concentrations of these acids have been used, although much weaker concentrations produce the reactions. The chromospectric expressions on filter paper among those compounds tested varied from cobalt blue to pinkish-gray. Nitrogenous and bromine derivatives tended to be of the latter sort.

In practice, the developed chromatogram has been treated in either of two ways to effect chromogenic definition of the spots. One utilizes reusable stocks, the other employs a mixed reagent that deteriorates in an hour or two upon standing. In the first method a stock spray or dip of indophenol blue (0.05 percent in 95 percent ethanol) is applied. After air-drying, the chromatogram is immersed in dilute organic acid with gentle agitation. The unfixed dye changes to a soluble yellow leachate and is extracted. Final elution is effected by washing with tap water to remove the acid. This intensifies spots and preserves their color. The stock acid solution is reusable, for the eluted dye is denatured shortly. In the single reagent method (*4*), indophenol blue is dissolved in concentrated formic acid in a ratio so that approximately a 0.05 percent dye solution is obtained when the acid is diluted to 20 percent with distilled water. Upon immersing the developed chromatogram in this dye solution, which is orange yellow, the fixation sites turn blue to pinkish-gray shades commensurate with the chromogenic nature of the reacting material. Leaching under running tap water removes the unfixed dye and acid.

The stability of spots by either method is good; there has been no measurable fading after 24 hours. Dipping in dilute ammonium hydroxide appears to accentuate spots even more and has aided in preserving color in the developed chromatograms. Chromatograms, however, do fade in time and under strong light, particularly if acid is not entirely eliminated.

The degree of halogenation of the aromatic base appears to have little bearing on color intensity. Color tone seems unrelated as well. For example, whereas dichloronaphthalene gives a relatively intense blue reaction, paradichlorobenzene is only moderately sensitive. Phygon (dichloronaphthoquinone) and Spergon (tetrachloro-*p*-benzoquinone) are very weakly sensitive. Furthermore, Aldrin (1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-endo, exo-5,8, dimethano-naphthalene) gives a more intense blue reaction than its exo-epoxide analog Dieldrin (1,2,3,4,10,10-hexachloro-exo-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo, exo-4,8-dimethano-naphthalene), which exhibits a blue-gray color. Dieldrin is less sensitive than Lindane (hexachlorocyclohexane), but both of these materials are far more sensitive than hexachlorobenzene. Yet, all three compounds incorporate six chlorine atoms on their aromatic bases.

Sensitivity apparently is not associated with saturation of the aromatic base, or the presence of oxy-groups. The unsaturated hydroxy-compounds pentachlorophenol or tetrachlorophenol, for example, stain more intensely than the saturated compound hexachlorocyclohexane and the unsaturated compounds hexachlorobenzene, pentachlorobenzene, and pentachloronitrobenzene. They also stain more intensely than Spergon, Phygon or Dieldrin, all of which are oxy-compounds. In contrast, the sulfone pesticide Tedion (tetrachloro-diphenyl-sulfone) stains very intensely. In fact, it is more sensitive than any other halogenated pesticide tested except DDT (dichloro-dephenyl-trichloroethane) which contains no oxygen group.

In general, compounds with more than one chlorine atom bonded to a given carbon atom stain more intensely than those with but one, particularly if the carbon atom is associated as a prosthetic group to the aromatic base.

Table 1 lists some of the more common chlorinated aromatics tested that are used as pesticides. The relative order of sensitivities is based on tests with available technical grade materials. The purities of some are in doubt, but the listed sensitivities offer an approximation of the efficacy of the reagent on a microgram per microliter basis (*5*).

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4. This method has given more uniform data. A preference is held for formic acid in the reagent, although lactic acid is quite suitable where ventilation is a problem.
5. Scientific paper No. 2277, Washington Agricultural Experiment Stations.

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