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Use of Factorial Design to Estimate Synergistic Effects in the Environment

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In environmental studies, experiments are often done under controlled conditions to develop a better understanding of how chemicals are transported and distributed. The ultimate goal is often the development of a model, conceptual and/or predictive. This is complicated by the fact that even in indoor environments, with their sharply defined boundaries, numerous variables need to be considered and, because of possible synergistic effects or interactions, they need to be considered simultaneously. Nevertheless, there is a tendency to make model investigations on a one-variable-at-a-time basis (Ozkaynak et al. 1982), assuming that the effects of the variables on the phenomenon of interest are additive, having a form such as

$$y = a + b_i x_i + b_2 x_2 + \ldots b_n x_n \ . \qquad (1)$$

Here, a relatively simple alternative approach is presented. It is based on the concept of factorial design (Box et al., 1978), which is used often in process development but has seldom been applied in environmental studies. It requires a small experimental effort if the number of variables is not too large. And since all the variables are considered simultaneously, not only can the main effects of the individual variables be estimated, but also the presence or absence of a synergistic effect can be detected.

The application of this approach was tested on a model study investigating the transfer of the biocide Lindane (γ-hexachlorocyclohexane) from a treated wood surface to cotton fabric. There is reason to believe that possible toxic effects are less likely to be caused by the incorporation of biocides through ambient air than by absorption from clothing or bedding which have adsorbed and concentrated these materials (Gebefügi et al. 1979 and 1984, Ruh et al. 1984).

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MATERIALS AND METHODS

The parameters investigated were air circulation, temperature, humidity, and amount of Lindane applied. A Lindane solution of 15 g/L in acetone was brushed on one side only of 16 different blocks of pine wood (15 cm x 15 cm x 2 cm) in such a way that one half of the blocks had a surface with 85 mg of Lindane while the remaining blocks had a surface with 255 mg of the biocide. These amounts are roughly comparable to the ones applied in the normal treatment of indoor wood surfaces. The acetone was allowed to evaporate overnight. This presumably also resulted in the loss of some of the applied Lindane, but since all of the blocks were treated in the same manner, this should have no effect on the results.

The treated wood was placed in aluminum boxes (50 cm on each side) with a glass top (Fig. 1). In the opposite corner was suspended a strip of cotton cloth (4 g, 13 cm x 24 cm). Humidity was kept either high or low by the introduction of a Petri dish, filled either with distilled water or dried silica gel. Temperature was controlled by placing the boxes in a phytotron (Vötsch) at either 18^{o} or 23^{o} C. Air circulation was produced by a small box fan (5 cm x 5 cm) with a capacity of 300 L/min. Each run lasted for 3 days.

At the end of this time, the cotton cloth was removed and extracted with 3 ml of hexane, and 2 µl of the extract were injected into a Carl Erba G1 gas chromatograph (isothermal 180^{o}) with a 1 m column packed with 2 % Silicon OV1 on Chromosorb E AW-DMCS, 80-100 mesh. The average result from all the runs was that 484.9 ng/g of Lindane sorbed on the cotton cloth after 3 days.

RESULTS AND DISCUSSION

In a general factorial design, a fixed number of levels for each variable is selected and experiments with all possible combinations run. This results in $l_1 \times l_2 \times \ldots l_n$ experiments, where l_i is the number of levels at each of n variables. The simplest factorial design is one, in which all variables are at two levels (high (+) and low (-)), resulting in 2^n experimental runs. For 4 variables, the pattern illustrated in Table 1 is used.

If the effects are additive, the only difference between y_1 and y_2, y_3 and y_4, etc. would be due to variable x_1 (air circulation). This readily gives an estimate

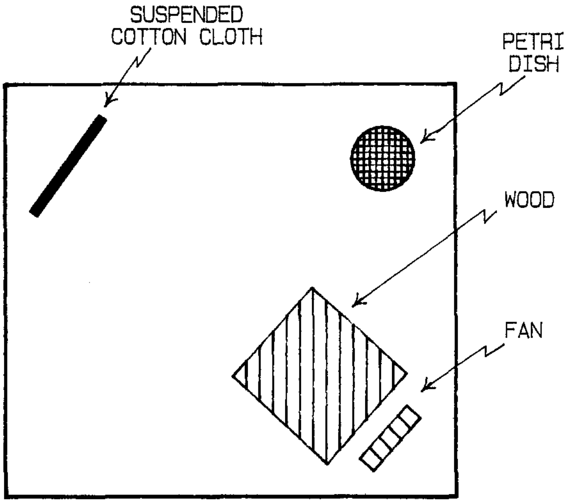

Figure 1. Schematic illustration showing set up of experiment

Table 1. Experimental design and results

Variable Levels C 1	H 2	A 3	T 4	Lindane sorbed on cotton	ng/g
-	-	-	-	y_1	558
+	-	-	-	y_2	401
-	+	-	-	y_3	319
+	+	-	-	y_4	302
-	-	+	-	y_5	785
+	-	+	-	y_6	631
-	+	+	-	y_7	408
+	+	+	-	y_8	609
-	-	-	+	y_9	369
+	-	-	+	y_{10}	321
-	+	-	+	y_{11}	265
+	+	-	+	y_{12}	495
-	-	+	+	y_{13}	625
+	-	+	+	y_{14}	435
-	+	+	+	y_{15}	497
+	+	+	+	y_{16}	739

C is the absence (-) or presence (+) of air circulation. H is low (-) or high (+) humidity. A is the amount of lindane applied, either 85 mg (-) or 255 mg (+). T is temperature of either 18^{o} C (-) or 23^{o} C (+)

of the main effect of x_1 on y (the amount of Lindane sorbed on cotton). Similarly, for x_2 (humidity), the main effect on y can be measured by the average differences between y_1 and y_3, y_2 and y_4, etc. The main effects for any other variables are calculated in a similar way.

According to Box et al. (1978), to secure the same precision for the estimation of the effect of a given variable, the one-factor-at-a-time experiment would need to employ 16 runs, 8 at each level with the other variables at some arbitrarily fixed level. In a similar manner, 3 further sets of 16 runs would be required to study the other 3 variables. In general, for n variables, the one-factor-at-a-time experiment requires n-fold more runs than the factorial experiment to obtain the same precision.

Therefore, the relative importance of each variable can be more efficiently estimated by the approach used here. However, the great value of the factorial approach is its use to estimate interactions (non-additivity) between variables. For example, with 4 variables, there is a total of 8 differences to measure the effect of x_1. However, 4 of these differences are determined at high values of x_2 and 4 at low ones.

x_2 (+)	x_2 (-)
$y_4 - y_3$	$y_2 - y_1$
$y_8 - y_7$	$y_6 - y_5$
$y_{12} - y_{11}$	$y_{10} - y_9$
$y_{16} - y_{15}$	$y_{14} - y_{13}$

If there is no interaction, there should be no significant difference between $y_4 - y_3 + y_8 - y_7 + y_{12} - y_{11} + y_{16} - y_{15}$ and $y_2 - y_1 + y_6 - y_5 + y_{10} - y_9 + y_{14} - y_{13}$. However, if there is a significant difference, it must mean that the effects of x_1 and x_2 are not additive and that an additive model would not be appropriate. In a similar manner, other interactions, including those between more than 2 variables, can also be investigated. The effects calculated for individual variables (main effects) and for multi-variable interactions are given in Table 2 and plotted as a frequency histgram in Fig. 2.

The effects of all variables that are only due to random error should have a roughly normal distribution. The effects calculated for variables which have a significant influence on the sorption of Lindane can be considered to be outliers from the normal distribution. There are various ways for testing whether or not a value is a significant outlier. The method used here is

Dixon's (1951). The results are given in Table 2 for effects, which have a probability < 0.05 that they belong to the normal distribution.

Not surprisingly, the amount of Lindane applied to the wood had a significant effect on the amount adsorbed on the cotton cloth. Tripling the amount applied increased the amount sorbed by 212.5 ng/g. Additional experiments with more levels would need to be run to determine the nature of the effect (linear, quadratic, etc.). However, the effect of the amount applied is independent of the other variables at the levels tested. Therefore, this variable could be included in a linear model.

Significant effects resulted from the interaction between air circulation and humidity and from the interaction between humidity and temperature. Changing the

Table 2. Effects of variables analyzed on lindane sorption

Variables	Mean Effect ng/g	Probability
Main Effects		
C	13	
H	-61	
A	212	< 0.005
T	-33	
Two Variable Interaction		
C x H	150	0.02 - 0.05
C x A	11	
C x T	45	
H x A	5	
H x T	122	0.02 - 0.05
A x T	-0	
Three Variable Interaction		
C x H x A	46	
C x H x T	26	
C x A x T	-43	
H x A x T	20	
Four Variable Interaction		
C x H x A x T	-7	

C = air circulation, H = humidity, A = amount of Lindane applied to wood, and T = temperature. Probabilities greater than 0.05 are not entered.

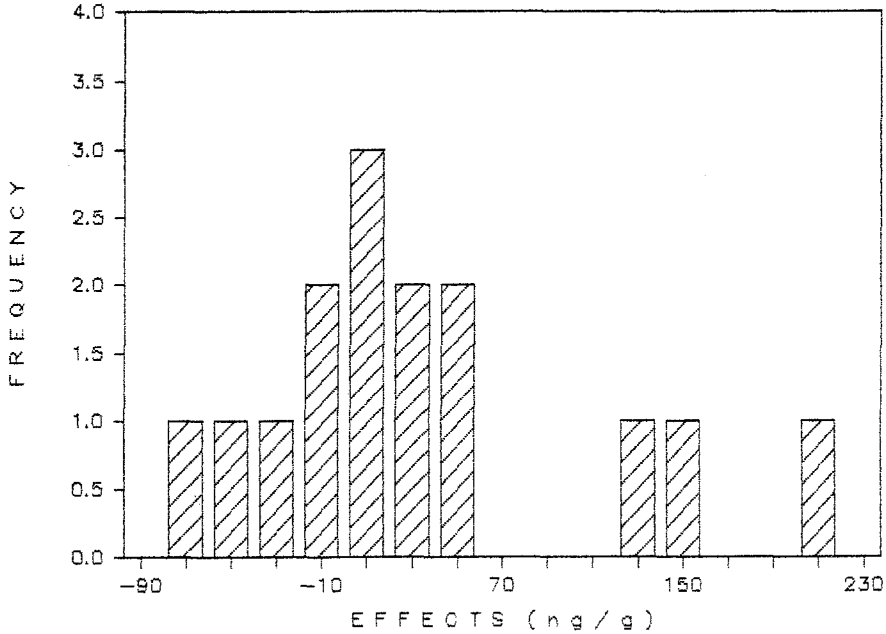

Figure 2. Frequency histogram of the magnitude of the effects of the parameters and their interactions on the sorption of Lindane on cotton cloth

level of these variables independently resulted in no significant effect. Air circulation only caused an increase in the amount of Lindane sorbed on cotton cloth when there was also an increase in humidity. The conclusion is that moisture serves as a carrier for Lindane. One can visualize Lindane's partitioning and transport as follows:

wood $\rightleftarrows$ vapor $\rightleftarrows$ cloth

Without the vapor, Lindane can not be readily removed from the wood. However, in the absence of air circulation, the Lindane containing vapor cannot reach the cloth efficiently. Therefore, an interaction is required, and a synergistic effect results. An additive model in the form of

$$y = a + b_1x_1 + b_2x_2 \qquad (2)$$

would not be appropriate, where y is the amount of Lindane sorbed on the cotton cloth, x_1 the amount of air circulation, x_2 the humidity, and a and b_i constants.

The significant effect caused by the interaction between temperature and humidity again points to the importance of humidity in the transport of Lindane. Presumably, temperature has an effect by regulating the amount of water that the air can accomodate. Higher temperature would also increase the rate of partitioning of lindane between the different compartments. However, the fact that temperature alone has no effect would indicate that this is unimportant under the conditions of the experiment done here. Apparantly at the end of 3 days, a steady state was reached, and the accelerated sorption of Lindane is offset by an equally accelerated desorption. The important point is that neither the effect of humidity nor that of temperature, in the range studied here, can be used in an additive model which assumes non-interaction between these two variables.

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