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PREDICTABILITY OF UNUSUALLY HIGH ACUTE TOXICITY TO PHOTOBACTERIUM PHOSPHOREUM OF 1,4-DI-SUBSTITUTED BENZENE DERIVATIVES

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MANAGEMENT PERSPECTIVE

Title: Predictability of unusually high accute toxicity to *Photobacterium phosphoreum* of 1,4-di-substituted benzene derivatives

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Date: 21 May 1987

This paper extends our earlier work on QSAR (quantitative structure-activity relationships) of mono- and 1,4-di-substituted benzene derivatives.

On the basis of measurements on over 100 of such benzene derivatives, we have now devised certain rules which will allow the computation of such toxicity values from the toxicities of certain related "base" compounds and structural properties that can either be measured or computed as well.

One important advance is the inclusion of previously excluded, highly toxic "outliers" in these rules, thereby providing a more generally applicable and more practical solution to the problem.

PERSPECTIVE-GESTION

Titre : Prévisibilité de la toxicité aiguë inhabituellement élevée pour Photobacterium phosphoreum des dérivés 1,4-disubstitués du benzène.

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Le présent document est la suite de nos travaux antérieurs portant sur les RQSA (rapport quantitatif structure-activité) des dérivés mono- et 1,4-disubstitués du benzène.

En se basant sur des mesures de plus de 100 de ces dérivés du benzène, nous avons pu mettre en évidence certaines règles qui permettent des calculs de valeurs de toxicité de ce type à partir des toxicités de certains composés apparentés "de base" et sur des propriétés structurelles qui peuvent être mesurées ou calculées.

Nous avons réalisé des progrès importants en intégrant au champ d'application de ces règles des cas d'exception fortement toxiques qui en étaient antérieurement exclus; ainsi, nous avons donc obtenu une solution plus générale et plus pratique à ce problème.

ABSTRACT

We report here on the relationships of the acute toxicities to *Photobacterium phosphoreum* of 1,4-di-substituted benzenes with their octanol/water partition coefficients and with the toxicities of the corresponding mono-substituted benzene derivatives and identify the types of derivatives for which such toxicities can or cannot safely be predicted from each other. "Outliers" to the previously established QSARs are identified and reasons for their aberrant behavior are discussed in terms of specific substituent groups and their electronic effects on the basic molecule and on the other substituents present, as measured by the substituent-induced chemical shifts of ^{13}C nuclear magnetic resonance data of the substituent-bearing carbon atoms of the benzene ring. With the combination of these parameters, the toxicity of highly toxic compounds can be predicted within an error of approximately 0.35 logarithmic toxicity units over more than 4 orders of magnitude. Nitrobenzene derivatives require the use of a parameter for the charge buildup on the oxygen atoms of the nitro group.

RESUME

Nous indiquons ici les relations de toxicité aiguë pour Photobacterium phosphoreum de benzènes 1,4-disubstitués, ainsi que leur coefficient de partage octanol/eau et les valeurs de toxicité de leurs dérivés monosubstitués correspondants, et nous identifions les types de dérivés pour lesquels de telles toxicités peuvent ou ne peuvent pas être prévues à un niveau de confiance élevé en les comparant les uns aux autres. Les "cas d'exception" au RQSA déjà définis sont identifiés et les raisons de leur comportement aberrant sont étudiées d'après leurs groupements substituants particuliers ainsi que leurs effets électroniques sur la molécule de base et sur d'autres substituants présents, d'après des mesures des déplacements chimiques induits par les substituants, mesurés à partir de données de résonnance magnétique nucléaire du C-13 d'atomes de carbone de l'anneau benzènique auquel sont fixés des substituants. En combinant ces paramètres, la toxicité des composés très toxiques peut être prévue avec une erreur d'environ 0,35 unité logarithmique de toxicité sur plus de quatre ordres de grandeur. Les dérivés du nitrobenzène nécessitent l'utilisation d'un paramètre pour l'accumulation de la charge sur les atomes oxygène du groupe nitro.

INTRODUCTION

Quantitative structure-activity relationships (QSARs) of physico-chemical characteristics with biological effects of organic chemicals are useful for the prediction of such effects for compounds and conditions which are not readily accessible to measurement. In particular, QSAR is evolving as an important tool for the screening of industrial chemicals for potentially hazardous or undesirable environmental properties and effects [1]. Although many compounds are amenable to such quantitative predictions, it is not unusual to find outliers whose effects, when measured, far exceed the values estimated from QSAR analysis. This "aberrant" behavior is usually explained - *a posteriori* - in terms of specific compound-active site interactions which are different from the normal interactions for which such QSAR models are valid. Until now, no mechanism has evolved to predict - *a priori* - which type of compounds are likely to exhibit effects outside these normal structure-activity relationships.

In recent publications [2,3] we have reported the acute toxicity of a large number of mono- and di-substituted benzene derivatives to *Photobacterium phosphoreum* (the Microtox^{TM} test). Satisfactory QSARs were derived but in both cases it was necessary to break down the original general group into subgroups, according to chemical similarities among the substituents, in order to achieve a reasonable degree of accuracy in predicting toxicity. For the 133 1,4-di-substituted benzene derivatives, a general dependence of acute toxicity on the octanol/water partition coefficient (log P) was found, but the standard deviation (s) was 0.75, which is too large to be of practical use. Consequently, this set was divided into subsets (1-R-C_6H_4-4-X) for which R was a constant (R= NH_2, NO_2, OH, Cl and $N(CH_3)_2$), but a satisfactory correlation between the acute toxicities of the di-substituted and the mono-substituted derivatives (C_6H_5-X) was found only for the series with R= Cl. After the elimination of 27 outliers from the general group, a highly significant correlation (s= 0.41) was found between the acute toxicities of the mono- and di-substituted derivatives. Inclusion of the octanol/water partition coefficient reduced the standard error to 0.34, which is a very acceptable value and is probably near the practical limit achievable for this type of data.

The problem of explaining and predicting the acute toxicities of the outliers remains, although the initial indications are that they occur for:

a) compound-specific toxicant-receptor interactions [4];

b) R,X pairs which can interact strongly (i.e., those with a large value of $sigma_p$, the Hammett sigma [5], or of the field (F) and resonance (R) parameters of Swain and Lupton [6].

However, there was no overall consistency between these latter parameters and the occurence of unexpectedly high acute toxicity. Therefore, additional parameters were sought for this purpose.

The chemical shifts in the NMR spectra of magnetic nuclei are influenced by molecular substituents, since the latter can alter the electron density in the vicinity of the magnetic nuclei and thus affect the amount of shielding experienced by these nuclei [7]. It is well-known that it is possible to correlate the substituent-induced chemical shift (SCS) in the ^{13}C NMR spectrum of substituted benzene rings with $sigma_I$ and $sigma_R$ the inductive and resonance parts of the Hammett sigma [8]. It is also well-known that large non-additive effects are observed for 1,4-di-substituted derivatives with strongly interacting groups [9] . The SCS values are, therefore, a direct experimental probe of the electronic interaction between substituents in 1,4-di-substituted benzene derivatives, and may be of use in the correlation and prediction of acute toxicities of these compounds. We have now performed such an investigation for both mono- and 1,4-di-substituted benzene derivatives, and the results are presented here.

MATERIALS AND METHODS

General

Most of the toxicity data used in these correlations has been published elsewhere [2,3], with the exception of the following derivatives of the general formula 1-R-C_6H_4-4-X, where R= CN, CF_3, F and some for R = Br, I. The test chemicals were purchased in the best grade available from Aldrich Chemical Co., Inc., or Fluka Chemical Corp. and were used without further purification, except where noted. The acute toxicities to *Photobacterium phosphoreum* were determined with the Mirotox^{TM} toxicity analyzer, following the procedure described previously. In some cases, up to 5% methanol was used to increase substrate solubility [10].

Experimental Values

All toxicity values reported here are the negative log to base ten ("p") values of the millimolar concentrations at which a 50% light reduction (Gamma = 1) was observed on 30 minute exposure. Each value is the mean of at least three independent determinations, usually performed with different bacterial suspensions to reduce any systematic variations or biases. The standard deviations of such triplicate analyses were normally in the order of 0.05 (logarithmic) toxicity units, and as high as 0.10 units for a few compounds of either high toxicity, sensitivity to

light, oxygen or water, or for those of very low solubility, where extrapolation to Gamma = 1 was necessary from lower Gamma values.

Parameters

Octanol/water partition coefficients (log P) were taken from the compilation by Hansch and Leo [11]. Where no experimental values were reported, log P was calculated from pi or fragment values and related compounds. If neither was available, log P was estimated on the basis of similar compounds' or groups' partition coefficients.

SCS values for C-1 and C-4 of mono- and di-substituted benzene deriva- tives were obtained from the literature for as many of the molecules studied in ref.2 and 3 as possible. The values reported here, (d1 and d4), give the 13C shifts at C-1 and C-4, respectively, relative to the 13C shift of benzene. In the regression analyses, these parameters were included independently, as well as their difference, (d1-d4), and the absolute value of their difference, (|d1-d4|). For some molecules, SCS values were reported by several authors, and most values agree to wthin 1%. Larger discrepancies were typically due to solvent or concentration effects. In these cases, the SCS measured in the most inert solvent, at the most dilute concentration, was used in the regression analysis.

The substituents' molar refractivity contributions, dMR_X, were also considered as parameters [11]. This parameter is commonly used in QSAR analysis as a measure of the molar volume of the substituent and, to a lesser degree, its hydrophobicity.

A detailed study of the relationship between the substituent-induced chemical shift in the ^{17}O NMR spectra of 1,4-di-substituted nitrobenzenes and the change in the electron population at that oxygen atom, as determined from *ab initio* molecular orbital calculations at the STO-3G level, has recently been published [12]. The absolute value of the latter values, (|dQox|), were also used as parameters for the nitrobenzene subset.

Table 1 lists the additional di-substituted chemicals investigated and for which SCS data were available with their acute toxicity values (pTm_R) and those of the corresponding mono-substituted compounds (pTm_H) [2], and their log P values. Table 2 gives the substituent-induced chemical shift data at C-1 (d1) and C-4 (d4), the dMR values and the |dQox| values, where available. There are in total 107 1,4-di-substituted benzene derivatives, and 39 mono-substituted derivatives. For the combined set of 93 di-substituted dervatives, there are 16 duplicate

entries, that is, the compounds appear in two subsets. All of the 1-R-C_6H_4-4-H compounds also appear in the mono-substituted series. For the combined halogen subset of 60 derivatives, there are 8 duplicate entries.

Computations

All toxicity value computations from the concentration/light reduction plots were done with the COMPUTOX™ program on an HP 86 computer. The statistical calculations were performed on the sam with pre-recorded linear and multiple regression analysis programs. Although there are duplicate entries, duplicate enumeration occurs only for the log P values.

RESULTS

From the previous studies on these benzene derivatives it has been concluded that:

1) In general the toxicity of the mono-substituted benzenes is a function of log P, while some outliers can be explained by the inclusion of other variables such as dMR_X; that is, they can be described by the general equation:

$$pTm_H = a + b \log P + c\, dMR_X \qquad (1)$$

2) After removal of the extreme outliers, the toxicity of the di-substituted benzene derivatives is a function of pTm_H and log P; that is, it can be described by the general equation:

$$pTm_R = a + b\, pTm_H + c \log P \qquad (2)$$

Since the data set to be investigated here is smaller than those in ref. 2 and 3, and since some new data is included, the dependence of pTm_R on these parameters was determined intially: to set a minimum standard for the quality of the correlations here, to identify any major differences with previous results and to identify the outliers. These results appear in Table 3, which also includes the correlation coefficients (r^2), the standard deviation (s) and the statistical F values for each data set or subset. (Note that the set with R= F was not included in the regression at this point.)

A better fit was then sought in an attempt to include the outliers. In the case of the nitrobenzene derivatives, the substituent-induced change in the electron population at the oxygen atom of some nitrobenzene derivatives, as determined by *ab initio* molecular orbital calculations was also included as a parameter where available. The relationships between pTm_R of the nitrobenzenes and pTm_H or |dQox| are shown in Figures 1 and 2. It has already been observed [3] that the chlorobenzene derivatives correlate very well with pTm_H alone. A larger set consisting of all compounds for which either R or X is a halogen was compiled from the data in Table 1 and 2 and a regression analysis was performed. Because of a similarity between these results and those of the CF_3 subset, the latter data were included in the halogen subset and the correlation was repeated. The best results from all of these correlations appear in Table 4 along with the statistical data as in Table 3.

DISCUSSION

The primary goal of the current investigation is to accurately predict the acute toxicities of compounds which have been found to be outliers in previous studies. However, as noted above, we must first establish a base correlation which will define the outliers. In earlier work, the toxicities of the 1,4-di-substituted benzenes were found to correlate generally with pTm_H and log P, equation (2). From the results in Table 3, it can be seen that the data set studied here retains this relationship. There is less dependence on log P than was found for the original set of 133 points, or for that set minus outliers (106 points). It should be noted however that of the original 133 points, 40 have a log P value of greater than 2.5, and 18 of these are absent from the present set. The same is true of the new data: for the CF_3, CN and F subsets, SCS data are not available for many substituents with log P>2.5. The reduced dependence of pTm_R on log P is thus due to the eliminaton of a disproportionate number of data points with large log P values. Apart from this change, the results are similar for the two data sets. The most significant parameter is still pTm_H, and while the overall correlaton is quite significant, the standard deviation is 0.71, which is not acceptable for practical purposes.

For the mono-substituted benzenes (R= H), the toxicity can be described by log P and dMR_X (dMR of substituent X) with a reasonable standard deviation of 0.38 and only 6 outliers. This correlation is improved slightly by the inclusion of d4, the SCS at C-4, but the improvement is not significant.

We have proceeded intuitively with the supposition that unexpectedly high toxicities are

associated with substituents which are capable of interacting strongly with each other through the benzene ring. The correlation between pTm_R and the other variables in Table 2 was determined for each subset. In every case but that of the NO_2 subset there is a significant dependence on d1, the substituent-induced chemical shift at C-1 of the benzene ring. There is also some dependence on pTm_H and dMR_X in most cases. Because the parameter pTm_H is itself dependent on dMR_X, it may be concluded that the latter is incorrectly weighted by the coefficient of pTm_H and must be altered. At the beginning of each regression calculation there was frequently some dependence on log P but this was typically eliminated upon the inclusion of pTm_H, which clearly acts as a "blanket" parameter by including all the factors which contribute to the toxicity of the mono-substituted species. The acute toxicity of the compounds in each subset, except for R= NO_2 and OH, follow the general equation:

$$pTm_R = a + b\ d1 + c\ pTm_H + d\ dMR_X \qquad (3)$$

From the data in Table 2, it is seen that the SCS values for C-1, when R= Cl, are quite small, and there is little variation regardless of the nature of the X substituent. While the magnitude of the SCS at C-1 is quite different for the other halogens, there is again little variation within the Br and F subsets. Moreover, the general degree of acute toxicity decreases in the order I > Br,Cl > F, as does the shift relative to benzene, d1. Because the regression equations for the halogens and for CF_3 were found to be fairly similar (3a-e), these subsets were combined into a single group of 60 data points. The best fit again follows the format of equation (3), but the dMR value of the group R, (dMR_R), is now included:

$$pTm_R = a + b\ d1 + c\ pTm_H + d\ dMR_X + e\ dMR_R \qquad (4)$$

It might have been anticipated that the molar refractivity contributions would be similar for both R and X, however, the dMR_X term is related to the pTm_H term, as noted above, and this affects the apparent dMR_X contribution. The standard deviation is accceptably small (0.35), and the actual error in calculating pTm_R is less than 0.6 logarithmic units for all but 5 of the 60 points. The five are: (F,NCS), (F,Cl), (CF_3,OH), (CF_3,NH_2) and (CF_3,NO_2). Only (F,NCS) and (CF_{OH}) are significantly more toxic than calculated. The OH, NH_2 and NO_2 subsets are found to correlate in a manner different from that of the halogens, *vide infra*, and in fact all three of these (CF_3,X) points are fit within the CF_3 subset, so the extension of the halogen set to include CF_3 may not be acceptable. The remaining outlier is (F,NCS). The phenylisothiocyanate (H,NCS) is quite toxic by itself, and may be one of the "strongly" interacting species, such as NO_2 is, but

there is insufficient data on this group to enable us to draw any conclusions.

As stated above, the other subsets correlate in a different fashion, but excellent correlations may be obtained in almost every case. The NH_2 subset is fit very well by d1 and pTm_H, with a small adjustment of dMR_X, equation 3f. There are no outliers in this subset; the highest observed residual is for X= CN, residual= 0.52.

The NO_2 subset does not correlate well with any of the original variables and the toxicity of the very toxic outliers, with X= NO_2, NH_2 and CN, cannot be predicted. Literature data is available for |dQox| at the oxygen atom for 9 of the 20 data points here (Table 2), including the outliers. It is found that the toxicity of all nine compounds is highly correlated with this parameter and pTm_R can be predicted with no outliers:

$$pTm_R = a + b\ |dQox| \qquad (5)$$

We can only conclude that this change renders the molecule more capable of reacting with receptors in the bacteria, however it would be most helpful to have more data to test this conclusion. The regression analysis shows that there is still a small dependence on dMR_X, which does improve the fit slightly, but, at this time, the need for this parameter is questionable, given the already small value of s, and the limited size of the physical dataset.

The OH subset still has outliers which cannot be resolved with the parameters here. The correlation given by equation 3g (Table 4)
has an unacceptably high standard deviation. In particular, the hydroquinone (OH,OH) compound has an unexpectedly high toxicity (pTm = 3.46) [3], which does not appear to be related to any of the parameters here. Similarly, the toxicity of the (OH, NHCOCH3) compound is unexpectedly low. It is necessary to remove these two ~~from the data set,~~ after which a fairly good correlation:

$$pTm_R = a + b\ d4 + c\ \log P \qquad (6)$$

predicts the toxicity of all but the (OH,CF_3) and (OH,H) compounds. The latter is overestimated. The toxicity of the (OH,CF_3) compound is fit adequately in the CF_3 subset.

The CN subset is fairly well fit with d1 and dMR_X, equation 3h, but on examination of the residuals, it is seen that the NO_2 point is not fit. This is one of the points which must be fit with |dQox| at the NO_2 oxygen, and therefore should be deleted from the CN set. Similarly, the (CN,F) point is too low, but is fit in the F subset, and is therefore also deleted. The

remaining points are fit with no outliers and an acceptable standard deviation of 0.45, equation 3i, (Table 4).

If the entire set of 93 data points (R= NH_2, NO_2, OH, Cl, CF_3, CN,) is fit as a group , the two most significant parameters are pTm_H and |d1-d4|. Although the dependence of pTm_R on d1 or d4 varies in sign and magnitude among the individual subsets, these differences are apparently accounted for in the general set by the combined term. Note that the parameter (d1-d4) was also tested and found to have no significance. The role of the |d1-d4| parameter is to measure the distortion or polarization of the electron distribution across the benzene ring. The change in the electron distribution causes the compound to be (presumably) more reactive, and therefore more toxic, however the reactive portion may be one or both of the substituents, or the ring itself. The value of log P is still found to have a small but significant effect on the toxicity. The acute toxicity is then described by the general equation:

$$pTm_R = a + b\,|d1-d4| + c\,pTm_H \qquad (7)$$

Two of the subsets can reasonably be removed from this correlation. The NO_2 subset appears to be dependent on the charge at the oxygen atom, which is not reflected by d1, d4, or |d1-d4|. The shift at d4, where X= F, is very high, in marked contrast to its relatively low toxicity. This is not a problem for the halogen subset, but is for the more general set, where the relevant parameter is not d1 but |d1-d4|. These data are also deleted, leaving a set of 80 points, which can be fit with an r^2 value of 0.51, and s= 0.50. The strongly interacting pairs (NH_2,CN) and (CF_3,OH) are also removed, as they are fit within their respective subsets, as is (OH, NHCOCH3). The remaining set of 76 points can be fit with r^2 = 0.57 and s= 0.41, equation 8, which is a highly significant relationship:

$$pTm_R = a + b\,|d1-d4| + c\,pTm_H + d\,\log P \qquad (8)$$

What we accomplish by these deletions is the removal of those data points for which there is an unusual electronic interaction between the substituents. As can be seen from the fit for the halogen subset, which contains five compounds of the type 1-R-C_6H_4-4-NO_2, it is not strictly necessary to eliminate the entire subset R= NO_2, but only those for which |dQox| is large, however, we eliminate all points for simplicity and consistency. The important results obtained here are that

1) equations 4, 7 and 8 are highly significant, and cover a broad range

of substituents and,

2) most of the "outliers" of the previous correlations have been included in one of the obtained correlations and can therefore be predicted with a high degree of confidence.

CONCLUSIONS

The present work demonstrates the predictability of highly toxic "outliers" from the previously observed relationships of the toxicities of 1,4-di-substituted benzenes (pTm_R) with those of the corresponding mono-substituted derivatives (pTm_H). The following rules can be made for the calculation of these toxicity values of mono- and di-substituted benzenes. If more than one of the specified groups is present, both equations should be tested, and the higher predicted toxicity should be assumed to be correct.

Mono-substituted benzenes --- equation 1

The results found here are similar to those of the earlier study [2]. Of the 38 mono-substituted benzenes, 36 could be fit by equation 1, Table 3, with a standard error of 0.38, and an r^2 value of 0.60, which is highly significant. The two derivatives which were omitted are X = para-C_6H_4-CN and $NHCOCH_3$. The first is really a 1,4-di-substituted benzene, and is fit in the CN subset. The toxicity of the second is quite low, and has been discussed previously [2].

Di-substituted benzenes --- General

1-R-C_6H_4-4-X --- equation 8

For compounds of the general formula 1-R-C_6H_4-4-X, acute toxicity is a function of pTm_H (the acute toxicity of the mono-substituted benzene, C_6H_5-X), |d1-d4| (the absolute value of the

difference in the substituent-induced chemical shift of C-1 and C-4 as measured by ^{13}C NMR), and the octanol/water partition coefficient. The role of the first parameter is self-explanatory, while the significance of the third parameter is widely documented. The second parameter is a measure of the change in the electron distribution across the ring, and indicates either an increased activity of one or both substituents or of the ring, due to a polarization of electronic charge. In certain special cases, given below, a more specific correlation may be appropriate.

Di-substituted benzenes --- Specific

1) X= Halogen or CF_3 --- equation 4d

Because of their chemical similarity, and a only minor dependence on electronic interactions, (|d1-d4|), the toxicity of these compounds may be determined from a simpler equation. However, d1 is still an important parameter, and the CF_3 data may not fit in cases where X is strongly interacting. In this event, the equation approprate for X should be used, if it exists, or the separate equation for the CF_3 group, equation 3e.

2) R= NO_2 --- equation 5

There is a good indication that the toxicity of the compound is related to the charge build up at the oxygen atom, as all 9 points for which these data are available could be fit, with no outliers, with this single parameter. More data for this correlation would be most useful to confirm this conclusion.

3) R= NH_2 --- equation 3f; R= CN --- equation 3i

For both of these sets, the parameters are the same as for the halogen subset but the coefficients vary in size and sign, reflecting the different degrees to which the toxicity is affected by these parameters. When either of these substituents is present, the appropriate subset equation should be used to predict the toxicity.

4) R= OH --- equation 6

There is still some uncertainty in the prediction of the toxicity of members of this subset, since the extreme outliers (OH,OH) and (OH,NHCOCH3) could not be fit with the available

parameters. The pH of the system, and the possibility of ion or zwitterion formation remain to be investigated. Further studies of this group are warranted.

5) R= NCS or NHCOCH3

The former appears to be more toxic than expected, the latter less toxic. The NCS group may possibly be another special case like NO_2 or OH, or may act by a specific mechanism [4], but insufficient data are available to confirm this at this time.

With the above guidelines, it is possible to predict the acute toxcity of all of the compounds studied, with no outliers, and a maximum standard error of the estimate of <0.4 logarithmic units. The present results confirm the supposition that the previous "outliers" occurred because of strong electronic interactions between the substituents. The actual toxicity is thus chiefly a function of an electronic parameter, d1 or |d1-d4|, and the "base" toxicity, pTm_H, of the corresponding mono-substituted benzene derivative. Consequently, also the toxicities of compounds with such strongly interacting substituents, for example, 1,4-dinitrobenzene, can be predicted from these equations.

These results raise some interesting questions. In particular, it appears that, at least within the mentioned subsets, there is no need to exclude any compounds on account of specific compound-receptor mechanisms. If this finding can be substantiated further, it is should also be investigated with respect to the effects of the same compounds on other organisms, and of more complicated compounds, such as multiple substituted benzene dervatives, on the same bacteria.

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Tab● 1. Toxicity values (pTm_R) of 1,4-di-substituted benzene derivatives of general formula 1-R-C_6H_4-4-X to Photobacterium phosphoreum, their CAS numbers, octanol/water partition coefficients (log P), and the toxicity values (pTm_H) of the corresponding mono-substituted benzene derivatives of the general formula C_6H_5-X, [2]a).

X	CAS #	pTm_R	pTm_H	log P
R= CF3				
Br	402-43-7	1.55	1.22	3.87
COCH3	728-86-9	1.40	0.89	2.46
CN	455-18-5	1.33	0.95	2.22
F	402-44-8	0.30	-0.28	3.15
R= ●				
C6H5	2920-38-9	2.99	1.91	3.38*
CH3	104-85-8	1.41	0.60	2.12
OCH3	874-90-8	1.35	0.76	1.54*
CF3	455-18-5	1.33	0.66	2.22
Br	623-00-7	1.32	1.22	2.42
CHO	105-07-7	1.02	1.34	0.91
COCH3	1443-80-7	0.45	0.89	1.01
F	1194-02-1	0.30	-0.28	1.70*
CN	623-26-7	0.26	0.95	0.99*
R= I				
OCH3	696-62-8	2.27	0.86	3.06*
COOH	619-58-9	0.45	0.86	3.23*

●

R= [redacted]

Br	106-37-6	1.96	1.22	3.85
OCH3	104-92-7	1.86	0.76	2.97
COCH3	99-90-1	1.52	0.89	2.44
F	460-00-4	0.78	-0.28	3.13

R= F

NCS	1544-68-9	2.28	1.81	3.42
COC6H5	345-83-5	1.57	1.31	3.32
Br	460-00-4	0.78	1.22	3.13
OCH3	459-60-9	0.76	0.76	2.25
CH3	352-32-9	0.46	0.60	2.88
COCH3	403-32-9	0.42	0.89	1.87
CHO	459-57-4	0.21	1.34	1.62
F	540-36-3	0.04	-0.22	2.41
CO[redacted]2	640-17-7	-0.31	0.31	0.91

) * indicates derived log P values; for details, see refs. [2,3].

Table 2. Substituent-induced chemical shifts on C-1 (d1) and C-4 (d4) of mono- and di-substituted benzene derivatives of the general formula 1-R-C_6H_4-4-X, with substituent molecular refractivity contribution (dMR_X), and, for nitro-substituted compounds, |dQox|, see text.

X	d1	d4	dMR_X	|dQox|	Reference
R = NH2					
CN	22.12	-28.5	6.33		9,13
CF3	21.04	-8.3	5.02		9,14
NO2	24.12	10.82	7.36		9
COCH3	22.77	-6.3	11.18		9
Cl	16.55	-5.19	6.03		9
C6H5	19.69	1.14	25.36		15
CH3	15.39	-0.58	6.65		9
COC6H5	23.09	-1.21	30.33		16
OC	11.56	24.38	7.87		9
NH2	10.24	10.24	5.42		9
F	14.05	27.97	0.92		9
H	17.91	-9.8	1.03		9
R = OH					
OH	20.93	20.93	2.85		17
I	25.63	-45.75	13.94		17
CF3	30.32	-5.57	5.02		17
CN	31.58	-24.98	6.33		17
C6H5	29.73	4.03	25.36		15
CH3	21.47	2.29	5.65		13,17,18
COCH3	32.29	2.02	11.18		17
COC6H5	32.69	0.99	30.33		16
Cl	25.63	-2.94	6.03		17
NO2	33.43	13.24	7.36		17
CH	32.89	2.02	6.88		17
F	24.17	28.18	0.92		17

H	27.50	-7.75	1.03		17
NHCOCH3	24.87	2.76	14.93		19
R = NO2					
NO2	22.72	22.72	7.36	108.5	9
NH2	10.82	24.12	5.42	81.7(avg)	9
CN	21.70	-10.02	6.33	73.3	9,13
I	18.99	-24.18	13.94		20
COC6H5	24.59	14.59	30.33		16
N(CH3)2	8.85	25.70	15.55		9
CHO	22.82	12.08	6.88	34.3	9
OCOCH3	16.94	26.99	12.47		25
OH	13.24	33.43	2.85	50.7	17
COOCH3	12.08	22.82	12.87		9
CF3	21.65	7.60	5.02	43.5	9,14
Br	18.74	1.65	8.88		9,14,21
CH3	17.81	17.58	5.65	15	9,13,14,18
COCH	21.59	7.79	6.96		24
OCH3	13.19	36.26	7.87		9
Cl	18.13	13.00	6.03		9
COCH3	22.04	13.06	11.88		9
H	19.95	6.18	1.03	0	9
CONH2	20.72	11.65	9.81		25
F	16.11	37.89	0.92	9.5	9,14
R = Cl					
NCS	4.60	1.68	17.24		26
COC6H5	10.49	1.89	30.33		14,16
I	5.87	-37.15	13.94		21
CH=CH2	5.22	7.38	10.99		27
OCH3	-2.81	29.85	7.87		9,14
CN	11.17	-17.55	6.33		9,13,14
Br	4.86	-7.28	8.88		9,14
Cl	4.22	4.22	6.03		9,14
NH2	-5.19	16.55	5.42		9

COOH	9.59	0.89	6.96	24
COCH3	11.07	6.99	11.18	9
CH3	2.72	7.87	5.65	9
OH	-2.94	25.63	2.85	17
CHO	12.67	9.95	6.88	9
CF3	9.81	0.77	5.02	9,14
H	5.93	-1.90	1.03	9
NO2	13.00	18.13	7.36	9
CONH2	7.71	4.66	9.81	20,25
NHCOCH3	-1.72	10.03	14.93	19
F	0.78	32.91	0.92	9,14

= N(CH3)2

CN	24.07	-31.07	6.33	9,13
NO2	25.70	8.85	7.36	9
H	22.28	-11.69	1.03	9,14

= CF3

OH	-5.57	24.68	2.85	17
NH2	-8.30	21.04	5.42	9,14
Br	1.41	-1.91	8.88	9,14
COCH3	6.02	11.31	11.88	9,14
CN	6.22	-12.27	6.33	9,14
NO2	7.60	21.65	7.36	9,14
Cl	0.77	9.81	6.03	9,14
H	2.31	3.67	1.03	9,14
F	-1.86	36.30	0.92	9,14

= CN

C6H5	-17.38	17.01	25.36	15
N(CH3)2	-31.07	24.07	15.55	9
NH2	-28.50	22.13	5.42	9
OH	-24.98	31.58	2.85	17
NO2	-10.02	21.70	7.36	9

Cl	-17.55	11.17	6.03	9,14,28
CH[illegible]	-19.10	15.30	5.65	9,14,18
OCH3	-24.44	34.45	7.87	9,14
CF3	-19.65	6.22	5.02	9,14
Br	-17.09	-0.36	8.88	9,14
CHO	-10.67	1.39	6.88	9
H	-15.96	4.35	1.03	9,14
COCH3	-11.91	11.69	11..88	13
F	-19.77	36.65	0.92	9,14
CN	-11.65	-11.65	6.33	9
R = I				
OH	-45.25	26.09	2.85	17
OCH3	-45.71	30.99	6.96	20
Cl	-37.15	5.87	6.03	21
H	-33.61	-1.01	1.03	22,23
NO2	-24.01	18.99	7.36	21
CO[illegible]	2.0	-27.41	7.87	24
R = Br				
Br	-7.28	-7.28	8.88	9,14
OCH3	-15.54	30.32	7.87	9,21
CF3	-1.91	1.41	5.02	9,14
COCH3	-0.04	7.43	11.18	9,21
Cl	-7.28	4.86	6.03	9,14
CN	-0.36	-17.09	6.33	9
H	-5.87	-1.51	1.03	9,14
NO2	1.65	18.74	7.36	9,14
F	-11.83	33.46	0.92	9,14
R = F				
NCS	32.75	-0.98	17.24	26
CO[illegible]5	37.19	5.49	30.33	16
Br	33.46	-11.83	8.88	9,14

OCH3	28.88	27.37	7.87	9,14
OH	28.68	24.67	2.85	17
CH3	31.71	4.96	5.65	9
COCH3	37.33	5.15	11.18	9
CF3	36.30	-1.86	5.02	9
CN	36.65	-19.77	6.33	9,14
CHO	38.17	4.65	6.88	9
NH2	27.97	14.05	5.42	9
NO2	37.89	16.11	7.36	9,14
F	29.44	29.44	0.92	9,14
Cl	32.91	0.78	6.03	9,14
H	34.79	-4.51	1.03	9,14
CONH2	35.55	2.37	9.81	25

R = H

C6H4-p-CN	0.39	10.70	30.66	16
C6H5	-0.31	9.99	25.36	20,23
C6H4-p-CH3	-1.31	12.69	29.98	16
C6H4-p-OH	-1.91	12.79	27.18	16
NCS	-1.05	2.91	17.24	26
I	-1.01	-33.61	13.94	29
OC6H5	-5.11	29.19	27.68	23
COOCH3	1.99	4.49	12.87	20
C6H4-p-NH2	-2.41	13.19	29.75	17
CHO	5.49	8.59	6.88	9,28
COC6H5	3.59	9.39	30.33	16
CH=CH2	-0.81	9.09	10.99	30
Br	-1.50	-5.87	8.88	9
OCOCH3	-2.31	22.99	12.47	23
COCl	6.19	4.59	10.44	23,28
CH2CH3	-2.81	15.89	10.30	20,29
Cl	-1.91	6.19	6.03	9,28
CN	4.49	-15.81	6.33	9,13
N(CH3)2	-11.81	22.59	15.55	9,23
NHCH3	-11.61	21.89	10.33	20
COCH3	4.19	8.69	11.18	9,23.28

COOH	5.39	2.09	6.96	20,24,28
CH2NH2	-1.81	15.09	9.09	20
NCO	-2.81	6.09	8.82	28
OCH3	-7.71	31.39	7.87	9,20.28
CF3	3.19	-9.01	5.02	9,28
CSNH2	2.76	11.11	18.28	25
OH	-7.31	26.89	2.85	17,28
CH3	-3.01	9.29	5.65	9,13
NO2	5.79	19.99	7.36	9,28
COCF3	6.69	-5.61	11.17	28
CONH2	2.81	5.87	9.81	25
NHNH2	-9.41	22.99	8.44	20
CH2OH	-1.31	12.29	7.19	23
NH2	-9.81	17.99	5.42	28
H	0.00	0.00	1.03	9
F	-4.51	34.79	0.92	9,28
NHCOCH3	-5.61	11.09	14.93	23

Table 3. Summary of multiple linear regression analyses of pTm_R according to equation (1) for mono-substituted benzenes and equation (2) for di-substituted benzenes.

R	Equation	Regression coefficients a	b (pTm_H)	c (log P)	n	r^2	s	F	Outliers (res.>0.7)
I	2a	3.081	-0.0002	-0.0432	6	.16	.99	.3	2
Br	2b	0.559	0.525	1.711	9	.59	.25	5.8	0
Cl	2c	0.310	0.715	0.164	20	.74	.33	24.4	0
F	2d	-0.747	0.693	0.309	16	.63	.44	11.2	2
CF3	2e	2.850	0.704	-0.660	9	.47	.69	2.7	2
NH2	2f	0.924	0.820	-0.186	12	.27	.77	1.7	3
	2g	1.251	1.072	-0.280	14	.34	.95	2.8	4
CN	2h	0.935	0.361	0.181	15	.10	.92	0.7	7
NO2	2i	1.259	0.412	-0.191	20	.09	.68	0.9	4
Halogen	2j	-0.348	0.463	0.422	51	.40	.61	16.2	10
Halogen+CF3	2k	-0.035	0.491	0.316	60	.30	.66	12.2	16
Total	2l	0.974	0.627	-0.044	93	.22	.71	13.0	26
Total-NO2,F	2m	0.585	0.467	0.167	76	.30	.51	15.9	11
H	1	0.029	0.038*	0.193	36	.60	.38	24.4	2

* This is the coefficient of dMR in equation (1).

Table 4. Summary of results from multiple linear regression analyses, for subsets or combined sets of data.

R	Equation	Regression coefficients *) a	b (dl)	c (pTm_H)	d (dMR_X)	e (dMR_R)	n	r^2	s	F	Outliers (res.>.7)
I	3a	0.512	-0.0445				6	.92	.27	43.1	0
Br	3b	0.890	-0.0254	0.627			9	.74	.22	8.4	0
Cl	3c	0.759	-0.0302	0.953			20	.78	.30	33.0	0
F	3d	2.540	-0.0883	0.692	0.0478		16	.81	.32	17.3	1
CF3	3e	2.537	-0.0940	1.040	-0.584**		9	.88	.45	12.3	0
NH2	3f	0.768	0.1037	1.357	-0.085		12	.78	.58	9.6	0
OH	3g	2.699	-0.059	1.425	-0.0608		14	.57	.80	4.4	4
CN	3h	-0.641	-0.083		0.0747		15	.64	.58	10.8	3
CN	3i	-0.969	-0.105		0.0637		13	.79	.45	18.6	1
Halogen	4a	0.737	-0.265	0.864			51	.79	.36	90.6	3
	4b	1.299	-0.0442	0.729	0.0288	-0.0931	51	.85	.35	66.0	0
(Halogen	4c	0.799	-0.0275	0.824			60	.73	.41	76.9	6
+ CF3)	4d	1.444	-0.0465	0.715	0.0281	-0.112	60	.80	.35	56.4	2
NO2	5	0.272	0.0240				9	.88	.35	49.2	0
OH	6	0.811	-0.0248	0.339			12	.60	.50	6.7	2
ll comp.	7	0.437	0.0215	0.677			93	.41	.61	31.7	23
ll comp.	8	0.115	0.0209	0.311	0.249		76	.57	.41	31.3	7
es NO2,F											

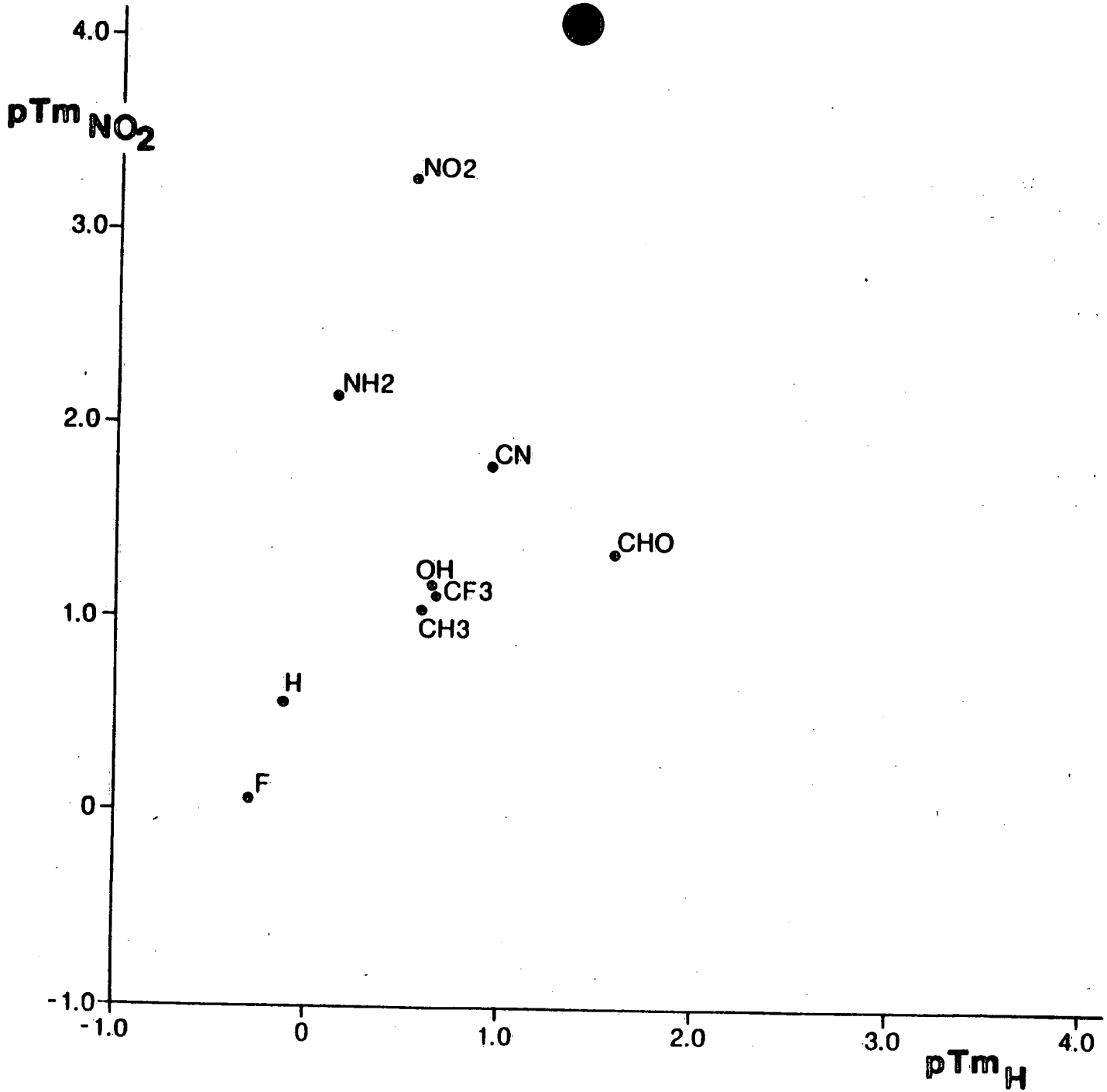
* Note that the terms in the column headings refer to equations 3 and 4 only, r description of independent variables in equations 5 to 8, see text.

** The parameter in this term is log P, not dMR_X.

FIGURE CAPTIONS

Figure 1. Toxicities (pTm_R) of nine p-nitro-substituted benzene derivatives versus the toxicities of the corresponding mono-substituted benzenes (pTm_H).

Figure 2. Toxicities (pTm_R) of nine p-nitro-substituted benzene derivatives versus the absolute values of the charge difference on the nitro group oxygen atoms (|dQox|).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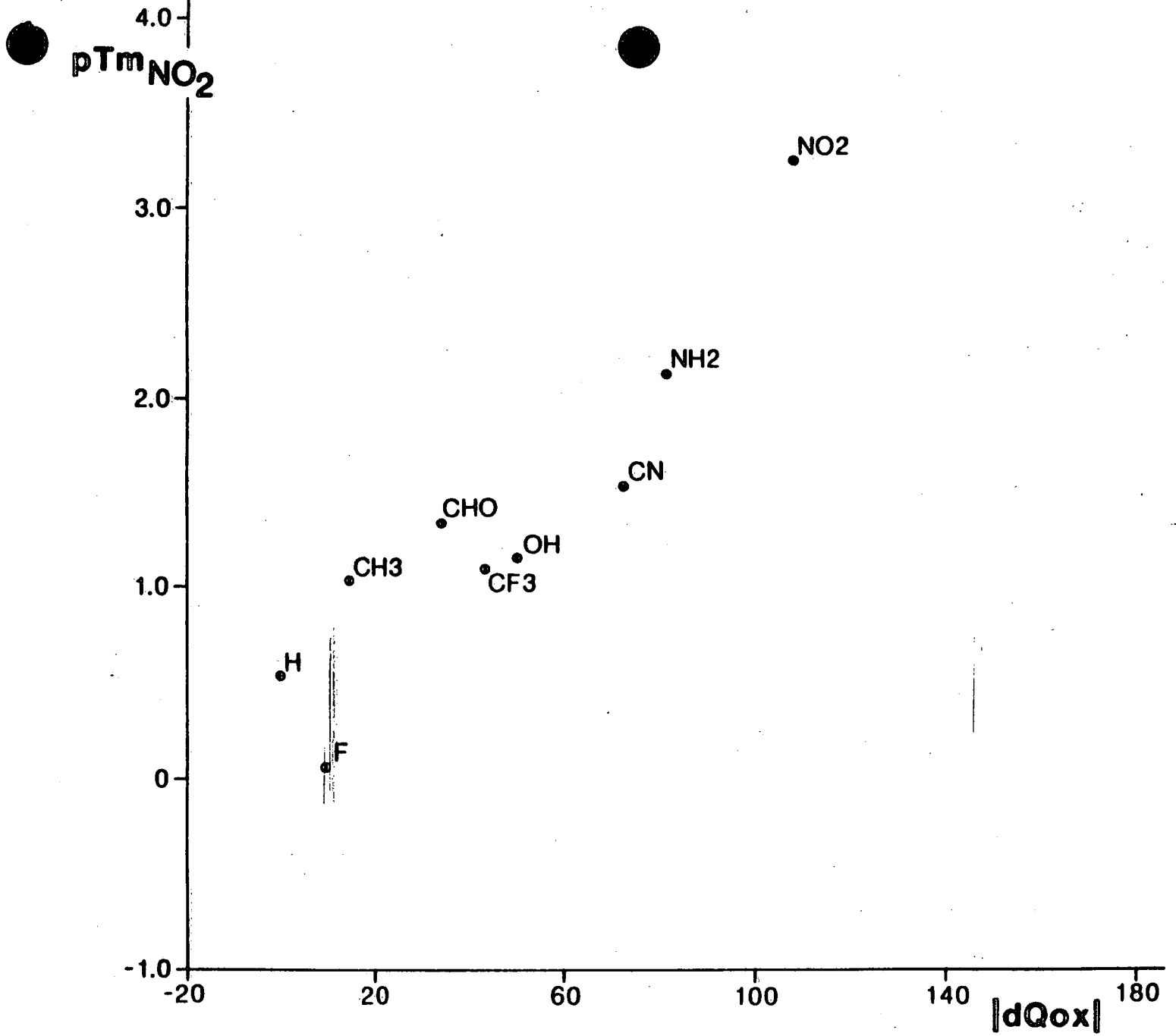
pTm NO2
3.0
2.0
1.0
0
-1.0
-1.0
0
1.0
2.0
3.0
4.0
pTm H
NO2
NH2
CN
CHO
OH
CF3
CH3
H
F

Fig. 1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3.0
2.0
1.0
0
-1.0
-20
20
60
100
140
180
|dQox|
NO2
NH2
CN
CHO
OH
CH3
CF3
H
F

Fig. 2