Quantitative Predictability of Carcinogenicity of the Covalent Binding Index of Chemicals to DNA: Comparison of the In Vivo and In Vitro Assays

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the fraction of nongenotoxic chemicals goes up in a data base the correlation between carcinogenicity and short-term tests goes down.

Studies of correlation between carcinogenic potency and quantitative degrees of response for positive short-term test data have given similar results (9–14). Again, a correlation is present, but relatively weak. For a summary of the quantitative correlation studies, see Table 2 in our previous paper (15). In that paper we also discussed the mathematical relationship between qualitative and quantitative correlation studies (15). The fact that a quantitative correlation is always present, even if weak, suggests that the additive role of promotion can attenuate, but not completely eliminate, the quantitative relationship existing between initiating potency and carcinogenic potency, provided that the bulk of the data base analyzed is not made up of pure, nongenotoxic chemicals.

As suggested above, differences between metabolism in the activation system of the short-term test and metabolism in the target cells for the initiation process could be a second major cause of the weak predictability of a given short-term test.

In the present study we tried to assess the relevance of metabolic activation in vivo in liver cells, versus an in vitro system of microsomes, again obtained from liver cells. The amount of DNA adducts was the common end point for both the in vivo and the in vitro assays considered, so the reason for a possible different degree of predictivity of the two tests had to be essentially related to the different metabolic activation. In addition, two other important factors could decrease the relationship between the in vivo and in vitro assays: first, DNA repair in the whole liver system may modulate the final DNA binding measured; second, there may be a difference in reactivity between chromosomal DNA and the purified DNA employed in the in vitro assay.

We had reason to suspect that a difference could indeed exist, considering the results obtained in several studies which we report below.

For instance, we compared the results obtained by Milsalis and Butterworth (16), who examined the autoradiographic repair induced by dimethylnitrosamine (DMN) in rat liver cells after treatment in vivo and also the results obtained by Williams and Laspia (17), who examined the autoradiographic repair induced by DMN in primary cultures of hepatocytes where DMN was already clearly active in vivo at $10^{-5}$ mole/kg, but active in vitro only at $10^{-2}$ M concentration.

In a paper by Kerklaan et al. (18), the induction of repairable DNA damage in E. coli cells evaluated in a host-mediated assay and in an in vitro assay were compared. The effects of DMN and 1,2-dimethylhydrazine (1,2-DMH) in E. coli cells recovered from the liver of injected mice were already clearly detectable at the dosage of 50 μmole/kg. In the in vitro assay (with the presence of mouse liver microsomes), DMN showed an extremely weak (not dose-dependent) response in a range of concentrations between $10^{-2}$ and $10^{-1}$ M; 1,2-DMH was completely inactive in the in vitro assay up to a concentration of 50 mM.

In our comparison between adduct formation in vivo and in vitro, the data available were mostly positive. Because the qualitative approach requires a balanced presence of positive and negative results, we were forced to choose a quantitative approach in our correlation study. We already used this methodology in several investigations (9–13). On the other hand, as a reference point, we had a paper by Lutz presenting a detailed quantitative study of the correlation between carcinogenicity and DNA adduct formation in vivo (14).

Methods

The criteria adopted for the selection and computation of the data and for their correlation studies are discussed in the following section.

Carcinogenic Potency

The carcinogenic potency data were mainly obtained from the data base of Gold et al. (19). We can expect a homogenous computation of potencies for all the chemicals listed in it. In this data base the carcinogenic potency is evaluated as TD$_{50}$ and is defined as “that chronic dose rate (in mg/kg body weight/day), which would halve the actuarially adjusted percentage of tumor-free animals at the end of a standard experiment time—the standard lifespan for the species” (20). For our computations, the TD$_{50}$ values were normalized in terms of μmole administered per kilogram body weight. For a minor group of chemicals, the above-mentioned data base was integrated with our own data (21). Our computations were brought in line with those of Gold et al. (19).

For each of the papers listed in the data base, we used the lowest reported TD$_{50}$ value (maximal oncogenic potency) where carcinogenicity was considered statistically significant ($p < 0.05$). When more than one paper was reported in the data base for the same chemical, one TD$_{50}$ value was selected from each of the papers. We then calculated the average of log TD$_{50}$.

Values of TD$_{50}$ for liver tumors were also considered for correlation studies. The liver was the organ where DNA adducts were measured.

Data were collected only from experiments on mice and rats after oral or parenteral administration. Only positive data were considered.

For our purposes, all the chemicals studied for the correlation between in vitro and in vivo DNA covalent binding and oncogenic potency had to be genotoxic. We defined as genotoxic the chemicals reported as positive in at least one-third of the genotoxicity tests considered in the data base of PfaFajda and Rosenkrantz (22). This partial arbitrary cut-off point was established in order to exclude a
study that could be suspected as promoting carcinogens more so than initiating carcinogens. One such example could be diethylstilbestrol. We are aware that this cut-off is an arbitrary one. However, it is well known that genotoxic chemicals are not positive in 100% of the tests. For instance, Ashby et al. estimated that nongenotoxic chemicals could be positive in about 20% of the tests, and very genotoxic chemicals could be positive in about 80% of the tests (29). Less potent genotoxic agents could give a response between 80 and 20%. The level selected was one considered capable of excluding a significant fraction of nongenotoxic chemicals without being too strict about the genotoxic efficiency required. We were not able to find a more objective threshold. On the other hand, too much time and detailed data was required to analyze the quality of each manuscript and, if necessary, to disagree with the results of the authors about the initiating potential of the chemicals considered.

**In Vivo DNA Covalent Binding (In Vivo-CBI)**

The data concerning the *in vivo* production of DNA adducts were obtained mostly from papers by Lutz (14, 24); some other data were obtained from the papers used for computing the *in vitro* DNA covalent binding index. According to Lutz (24), the *in vivo*-CBI was defined as:

\[
\text{micro mole chemical bound per mole nucleotides} / \text{millimole chemical administered per kilogram animal}
\]

The *in vivo*-CBI was computed for experiments on animals sacrificed 4 to 24 hr after treatment. This length of time is probably sufficient, usually being much longer than the biological half-life in *vivo* of most of the chemicals tested. In addition, the temporal range spanned by the different experiments can be considered sufficiently narrow with regard to the scale of CBI potencies. For this reason we did not deem it necessary to modify the *in vivo*-CBI formula proposed by Lutz (24).

The *in vivo*-CBI values concerned experiments on mice and rats treated by oral or parenteral routes. Only data on liver DNA adducts were collected. Only data concerning positive results were considered. When numerous data were available for the same chemical, only the first ten values were considered.

**In Vitro DNA Covalent Binding (In Vitro-CBI)**

The (*in vitro*-CBI) was defined as:

\[
\text{micro mole chemical bound per mole nucleotides} / \text{millimolar chemical in incubation mixture} * \text{incubation time in minutes}
\]

The *in vitro*-CBI values were computed from papers cited in the ICRDB Cancergrams (25); data were obtained also from Medline, Toxline, and Cancerline (National Library of Medicine, Bethesda, MD, USA; and Deutsches Institut fur Medizinische Dokumentation und Information (DIMDI), Cologne, FRG). When numerous data for the same chemical were available from different papers, only the first ten were used. From a mathematical point of view, it is extremely unlikely that the average of a random sample of 10 values should be totally unrelated to the entire population of all available data. As a consequence, we considered a sample size of 10 data to be adequate. The *in vitro*-CBI values were computed only from experiments in which the metabolic activation was obtained with microsomes prepared from the livers of mice or rats. Different activation systems were not considered. Data for seven directly alkylationg chemicals (incubations without microsomes) were also used. The considered experiments used native double-stranded calf thymus or salmon sperm DNA in the incubation mixtures. The molecular weight of the nucleotides was assumed to be equal to 309. In the same paper when more than one *in vitro*-CBI value was computable for the same chemical, only the highest CBI value was used. Only data concerning positive results were considered. Table 1 lists the chemicals for which we computed at least one *in vitro*-CBI value.

| *In vitro*-CBI | Animal species | Enzymatic induction | Concentration | Log<sub>10</sub> (in vitro-CBI) mean value | References |
|----------------|---------------|---------------------|---------------|------------------------------------------|------------|
| Aflatoxin B<sub>1</sub> | 1140 | R | PB | 1.0 | 2.85 | (26) |
| | 2575 | R | WI | e | (27) |
| | 162 | R | PB | 83 | (28) |
| | 490 | R | PB | 1.0 | 1.08 | (29) |
| | 843 | R | PB | 1.0 | (30) |
| | 1010 | R | PB | 1.1 | (31) |
| | 2960 | R | | e | (32) |
| | 175 | R | MC | f | (33) |
| | 403 | R | | g | (34) |
| Aflatoxin G<sub>1</sub> | 41.9 | R | PB | 83 | 1.62 | (28) |
| 3-Amino-1-methyl-5H-pyrido(4,3-b)-indole; (Trp-P-2) | 7.33 | R | MC | 1.0 | 0.826 | (35) |
| | 6.13 | R | PCB | | (36) |

(Continued on next page)
Table 1. (Continued)

| In vitro--CBI | Animal species | Enzymatic Log, (in vitro-CBI) | Concentration | \( \log_{10} \) (in vitro-CBI) mean value | References |
|---------------|----------------|-------------------------------|---------------|---------------------------------|------------|
| Benz(a)anthracene 0.605 | R | MC | 0.22 | -0.218 | (37) |
| Benzene 1.99 | M | PB | 0.67 | 0.299 | (38) |
| Benzo(a)pyrene 194 | R | MC | 1.37 | | |
| 20.1 | R | BF | 0.50 | | |
| 3.34 | R | 0.13 | | | |
| 49.2 | R | MC | g | | |
| 23.2 | R | MC | 0.20 | | |
| 1710 | R | MC | 2.0 | | |
| 40.0 | R | MC | h | | |
| 0.288 | R | MC | 0.22 | | |
| 3.17 | M | WI | 0.63 | | |
| 62.6 | R | MC | 0.36 | | |
| Bromoacetaldehyde 181 | M | 0.62 | 2.26 | | (46) |
| Bromobenzene 5.36 | M | PB | 0.67 | 0.729 | (47) |
| Bromoethanol 15.4 | M | 0.62 | 1.19 | | (48) |
| Carbon tetrachloride 0.110 | M | MC | i | -0.959 | (49) |
| Chlorobenzene 2.84 | R | PB | 0.67 | 0.453 | (50) |
| Cyclophosphamide 1.22 | R | PB | 0.25 | 0.0664 | (51) |
| Dibenzo(a,h)anthracene 0.192 | R | MC | 0.22 | -0.717 | (52) |
| 1,2-Dibromo-3-chloropropene 1.03 | R | MC | 0.02 | | |
| 1,2-Dibromomethane 6.65 | M | PB | 0.67 | 0.387 | (53) |
| 18.7 | M | PB | 0.67 | 0.582 | (54) |
| 8.02 | M | 0.62 | | | |
| 3.30 | M | 0.62 | | | |
| 0.263 | R | 2.2 | | | |
| 3,4’-Dichlorobenzidine 1.67 | R | 0.75 | 0.272 | | (55) |
| 1,1-Dichloroethane 1.20 | M | PB | 0.67 | 0.0792 | (56) |
| 1,2-Dichloroethane 8.74 | R | PB | 0.67 | 0.581 | (57) |
| 8.17 | M | PB | 0.67 | 0.582 | (58) |
| 0.827 | M | 0.62 | | | |
| Diethylnitrosamine 0.552 | R | PB | 1.6 | -0.258 | (59) |
| Diethylstilbestrol 1.33 | R | MC | 0.80 | 0.124 | (60) |
| 15,16-Dihydro-11-methylcyclopenta(a)phenanthren-17-one 24.1 | R | j | 1.38 | | (61) |
| 7,12-Dimethylbenz(a)anthracene 20.8 | R | PCB | 0.25 | 0.711 | (62) |
| 3.41 | R | PB | 1.0 | | |
| 3.22 | R | MC | 0.22 | | |
| 6.01 | R | MC | h | | |
| 2.60 | R | PCB | 1.0 | | |
| Dimethylnitrosamine 11.8 | R | WI | 1.6 | 0.792 | (63) |
| 6.39 | M | A | 4.0 | | |
| 3.17 | R | 1.6 | | | |
| Epichlorohydrin 0.155 | R | PB | 0.67 | -0.810 | (64) |
| Ethionine 0.162 | R | MC | i | -0.790 | (65) |
| α-Hexachlorocyclohexane 0.979 | M | PB | 0.20 | -0.00922 | (66) |
| γ-Hexachlorocyclohexane 1.49 | M | PB | 0.20 | 0.173 | (67) |
| Hexachloroethane 6.31 | M | PB | 0.67 | 0.800 | (68) |

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IN VIVO AND IN VITRO COVALENT BINDING

Table 1. (Continued)

| Liver microsomes | In vitro-CBI | Animal speciesa | Enzymatic inductionb | Concentration c | Log10 (in vitro-CBI) mean value | References |
|------------------|--------------|-----------------|---------------------|----------------|---------------------------------|------------|
| Isophosphamide   |              | R               | PB                  | k              | 1.34 (69)                       |            |
| 21.9             | M            |                 |                     |                |                                 |            |
| 7-Methylbenz(a)acridine | M       |                 |                     |                |                                 |            |
| 24.7             | R            |                 |                     |                |                                 |            |
| 3-Methylcholanthrene | R        |                 |                     |                |                                 |            |
| 13.6             | R            |                 |                     |                |                                 |            |
| 266              | R            |                 |                     |                |                                 |            |
| Mycophenolic acid| R            |                 |                     |                | 0.0453 (72)                    |            |
| 1.11             | R            |                 |                     |                |                                 |            |
| N-Nitrosopiperidine| R        |                 |                     |                | -0.772 (57)                    |            |
| 0.169            | M            |                 |                     |                |                                 |            |
| Pentachloroethene| M            |                 | PB                  | 0.67           | 1.11 (p)                       |            |
| 12.9             | R            |                 |                     |                |                                 |            |
| Quinoline        | R            |                 | PB                  | 1.6            | -0.772 (57)                    |            |
| 4.00             | R            |                 |                     |                |                                 |            |
| 1,1,2,2-Tetrachloroethylene | R            |                 |                     |                |                                 |            |
| 2.92             | M            |                 |                     |                |                                 |            |
| Tetrachloroethylene| R         |                 |                     |                |                                 |            |
| 3.74             | R            |                 |                     |                |                                 |            |
| 1,1,1-Trichloroethane | M        |                 |                     |                |                                 |            |
| 0.675            | R            |                 |                     |                |                                 |            |
| 1,1,2-Trichloroethane | M        |                 |                     |                |                                 |            |
| 16.4             | R            |                 |                     |                |                                 |            |
| Trichloroethylene| M            |                 | PB                  | 0.58           | -0.294 (78)                    |            |
| 0.316            | M            |                 |                     |                |                                 |            |
| 0.817            | M            |                 |                     |                |                                 |            |
| Diethyl sulfate  |              |                 |                     |                |                                 |            |
| 0.347            | M            |                 |                     |                |                                 |            |
| Dimethyl sulfate |              |                 |                     |                |                                 |            |
| 1.77             | R            |                 |                     |                |                                 |            |
| 1,1'-Ethylene-bis-(1-nitrosourea) | R |                 |                     |                |                                 |            |
| 2.06             | R            |                 |                     |                |                                 |            |
| Ethylmethane sulfonate | M  |                 |                     |                |                                 |            |
| 0.330            | R            |                 |                     |                |                                 |            |
| 0.238            | M            |                 |                     |                |                                 |            |
| 0.200            | M            |                 |                     |                |                                 |            |
| Ethyl nitrosourea|              |                 |                     |                |                                 |            |
| 0.129            | M            |                 |                     |                |                                 |            |
| 0.552            | M            |                 |                     |                |                                 |            |
| 1.53             | M            |                 |                     |                |                                 |            |
| Methylmethane sulfonate | M |                 |                     |                |                                 |            |
| 2.57             | M            |                 |                     |                |                                 |            |
| 2.69             | M            |                 |                     |                |                                 |            |
| Methyl nitrosourea|              |                 |                     |                |                                 |            |
| 4.12             | M            |                 |                     |                |                                 |            |
| 2.50             |               |                 |                     |                |                                 |            |
| 18.3             |               |                 |                     |                |                                 |            |
| Semicarbazide    |              |                 |                     |                |                                 |            |
| 0.00158          | M            |                 |                     |                |                                 |            |

*In vitro-CBI: in vitro DNA covalent binding index, as defined in "Methods."
*M, mouse; R, rat.
*A, acetone; BF, 5,6-benzoflavone; MC, 3-methylcholanthrene; PB, phenobarbital; PCB, polychlorinated biphenyls; WI, without induction.
*The concentration is expressed in mg protein/mL incubation mixture, unless otherwise stated.
*0.50 nmole P-450/mL incubation mixture.
*0.25 nmole P-450/mL incubation mixture.
*Microsomes from: 0.25; 0.7; 0.3; 0.14; 0.25 and 2.0 g of liver incubated in: 5, 6, 3, 3, and 50 mL of incubation mixture, respectively, for footnotes g, h, i, j, k, l.
*In the presence of cytosolic proteins.
*DNA source not specified.
*HeLa cell DNA.
*Our unpublished data.

Quantitative Correlation Studies

In a previous paper we showed that general sets of values concerning oncogenic potencies or short-term test potencies tend to display approximately a log-normal distribution (15). As a consequence, the correlation studies were made between log10 of potencies. When more than one potency value was available for the same compound, we used the average of log10 potencies. Usually, the clouds of points in graphs of log10 of potency X versus log10 of potency Y are also compatible with a linear regression analysis and parametric statistics (data not reported).
In order to determine the effects of a possible deviation from a log-normal distribution, the different correlations were also analyzed with the nonparametric Spearman's rank correlation coefficient, as reported in Tables 2 and 3.

Results

As detailed in the “Methods” section, the main conditions adopted for normalizing the data were studies on mice and rats treated by oral or parenteral routes for carcinogenicity data, on mouse and rat liver DNA for in vivo-CBI data, and using mouse or rat liver-metabolizing systems for in vitro-CBI data.

In these conditions, carcinogenicity data for 49 genotoxic chemicals (as defined in “Methods”) and in vivo-CBI data for 44 genotoxic chemicals were available. They had in vitro-CBI values spanning a range more than five logs10 wide, and totaled 48 compounds. However, 19 of them (i.e., chemicals No. 7-11, 17, 20, 21, 26-28, 30, 32, 34, 35, 39, 41, 43, 48) could not be used for any correlation because in vivo data were either unavailable or the chemicals were not defined as genotoxic compounds. Thus, we were left with 29 useful compounds. In Table 1 both the in vitro-CBI values for each experiment and average log_{10} (in vitro-CBI) values were reported; however, for correlation studies only the average log_{10} values were used.

We wanted to consider only genotoxic compounds. The importance of this point may be illustrated by considering that during their lifetime animals subjected to chronic carcinogenicity experiments with promoters can be exposed to a background level of initiating events that are sufficient to combine with the effects of a full-promoting treatment, thus generating a detectable tumor incidence. As a consequence, even purely or prevalently promoting agents can give positive results in chronic carcinogenicity experiments in rodents. However, a study of the correlation between DNA adduct formation and carcinogenicity makes sense only if we deal with initiating agents.

In order to increase the probability that we were dealing with an initiator and not a promoter, we established the condition that a chemical could be used in our correlation study only if it was positive in at least one out of three genotoxicity tests, as judged

Table 2. Quantitative correlations among overall carcinogenicity (TD\textsubscript{90}), in vivo DNA covalent binding (in vivo-CBI), and in vitro DNA covalent binding (in vitro-CBI).\textsuperscript{*}

| Couple of parameters | No. of chemicals | Correlation coefficient, \( r^p \) | Correlation coefficient per ranks, \( r^p \) |
|---------------------|------------------|-----------------|-----------------|
| Overall correlation |                  |                 |                 |
| \( \log (\text{in vitro-CBI}) = f (\log (\text{TD}_{90})) \) | 26               | 0.44            | 0.30            |
| \( \log (\text{in vivo-CBI}) = f (\log (\text{TD}_{90})) \) | 41               | 0.52            | 0.51            |
| \( \log (\text{in vitro-CBI}) = f (\log (\text{in vivo-CBI})) \) | 21               | 0.64            | 0.53            |
| Correlation for the same 18 chemicals |                  |                 |                 |
| \( \log (\text{in vitro-CBI}) = f (\log (\text{TD}_{90})) \) | 18               | 0.42\textsuperscript{a} | 0.27            |
| \( \log (\text{in vivo-CBI}) = f (\log (\text{TD}_{90})) \) | 18               | 0.46\textsuperscript{a} | 0.36            |
| \( \log (\text{in vitro-CBI}) = f (\log (\text{in vivo-CBI})) \) | 18               | 0.58\textsuperscript{a} | 0.47            |

\textsuperscript{*}The definitions of the parameters are reported in “Methods.” In vitro-CBI data were obtained from the data base listed in Table 1. In vivo-CBI data were obtained from (14,24,28,33,15,50,53,65,66,68,74-76); our unpublished data were used for pentachloroethylene. TD\textsubscript{90} were obtained from Gold et al. and Parodi et al. (19,21).

\textsuperscript{a}Parametric statistical computations according to Snedecor and Cohran (87). The \( r \) values are statistically different from zero with a \( p < 0.05 \) except where otherwise specified.

\textsuperscript{b}Nonparametric statistical computations according to Siegel (88). \( r^p \) = nonparametric correlation coefficient according to Spearman.

Table 3. Quantitative correlations among liver carcinogenicity (TD\textsubscript{90}), in vivo DNA covalent binding (in vivo-CBI), and in vitro DNA covalent binding (in vitro-CBI).\textsuperscript{*}

| Couple of parameters | No. of chemicals | Correlation coefficient, \( r^p \) | Correlation coefficient per ranks, \( r^p \) |
|---------------------|------------------|-----------------|-----------------|
| Overall correlation |                  |                 |                 |
| \( \log (\text{in vitro-CBI}) = f (\log (\text{TD}_{90})) \) | 13               | 0.64            | 0.55            |
| \( \log (\text{in vivo-CBI}) = f (\log (\text{TD}_{90})) \) | 25               | 0.57            | 0.63            |
| Correlation for the same nine chemicals |                  |                 |                 |
| \( \log (\text{in vitro-CBI}) = f (\log (\text{TD}_{90})) \) | 9                | 0.66\textsuperscript{a} | 0.50            |
| \( \log (\text{in vivo-CBI}) = f (\log (\text{TD}_{90})) \) | 9                | 0.75\textsuperscript{a} | 0.85            |

\textsuperscript{*}The definitions of the parameters are reported in “Methods.” In vitro-CBI data were obtained from the data base listed in Table 1. In vivo-CBI data were obtained from (14,24,28,33,15,50,53,65,66,68,74-76); our unpublished data were used for pentachloroethylene. TD\textsubscript{90} were obtained from Gold et al. and Parodi et al. (19,21).

\textsuperscript{a}Parametric statistical computations according to Snedecor and Cohran (87). The \( r \) values are statistically different from zero with a \( p < 0.05 \) except where otherwise specified.

\textsuperscript{b}Nonparametric statistical computations according to Siegel (88). \( r^p \) = nonparametric correlation coefficient according to Spearman.
from the data base of Rosenkranz and Palajda (22).

We analyzed the distribution of the mean values of 
\( \log_{10}(\text{in vitro-CBI}) \) concerning the 48 chemicals listed in Table 1. As shown by the histogram in Figure 1, these resulting values were approximately log-normally distributed, in accordance with our previous observations (15). As a consequence of this result, we considered it acceptable to apply parametric statistics to \( \log_{10} \) of potencies.

Table 1 also details the data base of the \textit{in vitro-CBI} values we computed, and gives an idea of the differences in experimental conditions present in the \textit{in vitro} experiments. Although we normalized the results for drug concentration and incubation time, from the point of view of metabolic activation, the experimental conditions were definitely less homogenous than those for the \textit{in vivo} experiments. However, as the reader will see in the following analysis, the additional statistical noise brought about by this lack of data homogeneity apparently did not play an important role. As can be seen in Table 1, for 57 out of the 87 experiments listed, it was possible to normalize the data for microsomal protein concentration. By calculating the correlation between normalized and nonnormalized data, we found \( r = 0.87 \), suggesting that the lack of this normalization is not terribly disruptive. On the other hand, by using only normalized data, we would have impoverished our data set too much.

With the data we collected, it was possible to study the quantitative correlation with carcinogenicity of the \textit{in vitro-CBI} for an overall group of 26 genotoxic chemicals, and the \textit{in vivo-CBI} for an overall group of 41. A correlation study with more homogeneous data was possible with 18 chemicals where triplets of data on carcinogenicity, \textit{in vivo} and \textit{in vitro-CBI}, were available. The results are reported in Table 2.

No statistically significant differences were observed in predictivity of carcinogenicity after discarding from the correlation four directly alkylating chemicals (i.e., chemicals No. 44-47 of Table 1), whose data were available both for \textit{in vitro-CBI} and \textit{in vivo-CBI}.

As previously reported, quantitative correlation levels with carcinogenicity around 0.4-0.5 are very common in most of the short-term tests (15). In this respect the results reported in Table 2 seem to suggest that DNA adduct formation can contribute to our knowledge of genotoxicity and the initiating potential of a given chemical with a degree of efficacy similar to that of the most common short-term tests. This conviction is reinforced by the fact that the differences in quantitative predictivity previously found for different short-term tests never reach a level of clear statistical significance (15).

In a recent paper, Lutz reported a correlation level with carcinogenicity of the \textit{in vivo-CBI} with \( r = 0.81 \) (14). Compared with our \( r = 0.52 \), this difference is statistically significant \((p=0.03)\). However, it has to be remembered that we considered 12 more chemicals in addition to the 29 considered by Lutz, and we also considered additional experiments for those same 29 chemicals. Moreover, Lutz considered only carcinogenicity results obtained in the same species used for DNA adducts.

The results obtained with the Spearman's test were not very different from those obtained with the parametric approach. The small difference observed is probably related more to the small size of the set considered than to an important systematic deviation from normality of the log of potencies of the general population of data.

As reported in Table 2, we have also investigated the quantitative correlation existing between \textit{in vitro-CBI} and \textit{in vivo-CBI} data. The correlation coefficient with \( r \) value around 0.6 found in this study appears relatively high if compared with \( r \) values around 0.4, previously found when comparing different short-term tests with one another (9,10). This finding confirms that the highest correlation is reached with tests that use the same biological endpoint. As an example, in a previous study, the \textit{in vivo} alkaline DNA elution test, which looks at the endpoint DNA damage, appeared to be correlated with the \textit{in vivo-CBI} by \( r = 0.66 \). By contrast, it was correlated by lower \( r \) values around 0.3, with tests that use different endpoints such as Ames' test and \textit{in vitro} cell transformation test (9).

In Table 3 we reported the correlation between DNA adducts and carcinogenicity in the liver. In this case the sample size becomes further reduced. The differences between \( r \) values obtained for overall tumors and the \( r \) values obtained for liver tumors are not statistically significant. However, a general trend seems to emerge suggesting that the identity of the target organ perhaps plays some role in determining...
the correlation level. Even in this case, the predictivities of in vitro-CBI and in vivo-CBI are very similar.

**Discussion**

*In vivo* DNA binding has already been proposed by Lutz (14, 21) as a valid short-term test for assessing genotoxicity.

To our knowledge, this is the first time that *in vivo* and *in vitro* covalent bindings have been compared in terms of their predictivity of carcinogenicity. *In vitro* tests are usually less expensive and more rapid than *in vivo* tests. In addition, the use of *in vitro* tests can significantly reduce the number of animals used to assess the initiating potential of a given chemical as a carcinogen. On the other hand, the general knowledge that we have about absorption, metabolism, and catabolism of chemicals leads us to suspect that *in vivo* assays could be more predictive than *in vitro* assays.

As a matter of fact, the papers mentioned in the “Introduction” (16-18) seem to suggest that for DMN and 1,2-DMH, two important classical initiating carcinogens, the *in vivo* assay is indeed much better than the *in vitro* assay.

However, this difference is no longer evident when we look at the globality of the 30 to 40 chemicals that we have examined in this work. Admittedly, the sensitivity of the experimental approach adopted for comparing *in vitro* and *in vivo* predictivity is not very great (a larger data base was not available); hence, it remains possible that the *in vivo* assay is slightly more predictive than the *in vitro* assay, as suggested by the small differences in correlation coefficients found in our results. We have to stress that only limited conclusions can be justified by a data base of the size mentioned above.

In a recent study Tennant et al. (5) found the qualitative correlation between the carcinogenicity in mice and rats to be only 67%. Given that even the level of this type of internal correlation is limited, we cannot expect especially high correlation levels between short-term tests and carcinogenicity. In this perspective the levels of predictivity found in our study can be considered to be reasonably good.

If we compare the *in vitro*-CBI evaluation (in the presence of liver microsome activation) with other tests, the quantitative predictivity of this assay seems to be similar to that of other classical short-term tests, such as Ames' test, SCEs in mouse bone marrow, morphological transformation in *in vitro*, DNA fragmentation in *in vivo* (liver), and DNA repair in *in vitro* (hepatocytes) (15).

In conclusion, *in vitro*-CBI seems to have a predictivity similar to most short-term tests and is not clearly inferior to *in vivo*-CBI. We think that *in vitro*-CBI can be proposed as another short-term test in its own right, especially in the perspective of reducing the number of animals used in toxicity studies.

Our data could also suggest that for other types of short-term tests (mutagenicity and chromosomal damage), *in vivo* versions of the tests are not necessarily dramatically more predictive than the corresponding version *in vitro* (with liver microsome activation). This could be a subject which deserves further investigation.

Finally, it could be interesting to compare *in vitro* and *in vivo*-CBI for a homogeneous class of chemicals. In this respect we have started investigation with the family of chloroethanes.

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