different animal sources[18-20]. Experiments in order to demonstrate the capacity of the Sertoli cells to synthesize pregnenolone and P by cholesterol are in progress and the results will be reported in a forthcoming paper.

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DISPUTANDUM

Theoretical prediction of carcinogenicity: Quasi-quantification by quasi-valence[1]. A reply to V. Veljkovic and D.I. Lalovic

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Summary. We have shown the recently proposed method for prediction of carcinogenicity by 'average quasi-valence number' to be neither a good predictor of carcinogenicity, nor of non-carcinogenicity.

Veljkovic and Lalovic have recently proposed that a quantity termed the 'average quasi-valence number' can be used as a predictor of carcinogenicity[2]. This is defined as:

$$Z^* = \sum_{i=1}^{m} N_i Z_i \Big/ \sum_{i=1}^{m} N_i,$$

where N_i is the number of atoms of the i^{th} kind present in a molecule, Z_i is the number of valence electrons in the i^{th} element (except for the halogens where $Z=1$ rather than $Z=7$ is used) and m is the number of elements present in the molecule. As an example, the molecular formula for carbon tetrachloride is CCl_4. Consequently:

$$Z^* = \frac{(1\times 4)+(4\times 1)}{5} = {}^{8}/_{5} = 1.60$$

The authors state that a low value for Z* is necessary but not sufficient for carcinogenicity, while a high value is both necessary and sufficient to indicate non-carcinogenicity. The borderline between these 2 states is supposed, on empirical evidence, to occur at $Z^* = 3.2$. Before such a method is accepted as valid, it is reasonable to expect that it satisfy the following criteria. 1. Few predictions of carcinogenicity when the molecule is non-carcinogenic (false positives) or predictions of non-carcinogenicity when the molecule is carcinogenic (false negatives). 2. A correlation between Z* value and carcinogenic potency. 3. A large change in Z* value between a procarcinogen and the activated, ultimate carcinogen.

False positives and false negatives. The table illustrates that method of average quasi-valence number misclassifies many compounds. False positives include cyclohexane, ethyl alcohol and glucose. False negatives are acetylaminofluorene-N-sulfate (the ultimate carcinogen after activation of 2-acetylaminofluorene), the ultimate metabolite of benzo(a)pyrene, 4-NQO and all but one of the aflatoxins and the nitrosoguanidines. Such glaring inconsistencies in themselves are enough to raise grave doubts about the ability of average quasi-valence number to predict carcinogenicity. Our list, however, shows that average quasi-valence number does not even fulfill the criteria set up by the authors in that a value of $Z^* > 3.2$ is not necessary and sufficient for non-carcinogenicity. Moreover, if the entire list is considered, it can be seen that there is no correlation of Z* with carcinogenicity. Carcinogens and non-carcinogens are found throughout the entire range of Z* values. It is true that the authors claim only that Z* is necessary but not

Average quasi-valence number and carcinogenicity of some organic compounds

Compound	Molecular formula	Z*	Carcinogenicity[a]	Reference
Carbon tetrachloride	CCl_4	1.60	+	9
Propane	C_3H_8	1.82	−	11
Butane	C_4H_{10}	1.86	−	11
Vinyl chloride	C_2H_3Cl	2.00	+	9
Nitrogen mustard	$C_5H_{11}NCl_2$	2.00	+	9
Cyclohexane	C_6H_{12}	2.00	0	9
N-Butyl alcohol	$C_4H_{10}O$	2.13	0	9
bis-Chloromethyl methyl ether	C_2H_5ClO	2.22	+	9
Ethyl alcohol	C_2H_6O	2.22	0	9
Ethyleneimine	C_2H_5N	2.25	+	9
Acetone	C_3H_6O	2.40	0	9
Diethylnitrosamine	$C_4H_{10}ON_2$	2.47	+	9
Dimethyl sulfoxide	C_2H_6OS	2.60	0	9
4-4′-Methylene-bis-2-chloroaniline (MOCA)	$C_{13}H_{12}N_2Cl_2$	2.62	+	3
Napthalene	$C_{10}H_8$	2.67	0	9
4-Aminobiphenyl	$C_{12}H_{11}N$	2.67	+	4
2-Aminobiphenyl	$C_{12}H_{11}N$	2.67	0	4
Benzidine	$C_{12}H_{12}N_2$	2.69	+	3
α-Napthylamine	$C_{10}H_9N$	2.70	0	4
β-Napthylamine	$C_{10}H_9N$	2.70	+	4
Dimethylnitrosamine	$C_2H_6N_2O$	2.73	+	9
Anthracene	$C_{14}H_{10}$	2.75	0	9
Phenanthrene	$C_{14}H_{10}$	2.75	0	9
Ethyl carbamate	$C_3H_7O_2N$	2.77	+	9
1-Aminoanthracene	$C_{14}H_{11}N$	2.77	+	4,9
2-Aminoanthracene	$C_{14}H_{11}N$	2.77	+	4,9
2-Acetylaminofluorene	$C_{15}H_{13}NO$	2.80	+	9
4-Acetylaminofluorene	$C_{15}H_{13}NO$	2.80	0	9
Safrole	$C_{10}H_{10}O_2$	2.82	+	9
Vinyl acetate	$C_4H_6O_2$	2.83	0	9
Dibenz(a,h)anthracene	$C_{22}H_{14}$	2.83	+	3
Pyrene	$C_{16}H_{10}$	2.85	0	9
Benzo(a)pyrene	$C_{20}H_{12}$	2.88	+	9
Ethyl methanesulfonate	$C_3H_8O_3S$	2.93	+	9
1′-Hydroxysafrole	$C_{10}H_{10}O_3$	2.96	+	9
Diphenylnitrosamine	$C_{12}H_{10}N_2O$	2.96	0	9
Acetic acid	$C_2H_4O_2$	3.00	0	9
1′-Acetoxysafrole	$C_{12}H_{12}O_4$	3.00	+	9
Methyl carbamate	$C_2H_5O_2N$	3.00	0	9
Glucose	$C_6H_{12}O_6$	3.00	0	10
Cycasin	$C_8H_{16}N_2O_7$	3.03	+	9
Methylazoxymethanol	$C_4H_8N_2O_3$	3.06	+	9
Benzo(a)pyrene-4,5-epoxide	$C_{20}H_{10}O$	3.10	+	6
Methyl methanesulfonate	$C_2H_6O_3S$	3.17	+	9
1,3-Propanesultone	$C_3H_6O_3S$	3.23	+	3
Acetylaminofluorene-N-sulfate	$C_{15}H_{12}O_4NS$	3.24	+	4,5
Aflatoxin G_2	$C_{17}H_{14}O_7$	3.26	0?	9
Benzo(a)pyrene-7,8-diol-9,10-epoxide	$C_{20}H_{10}O_3$	3.27	+	6
Aflatoxin B_1	$C_{17}H_{12}O_6$	3.31	+	9
Aflatoxin G_1	$C_{17}H_{12}O_7$	3.39	+	9
Sterigmatocystin	$C_{18}H_{12}O_6$	3.40	+	9
N-Ethyl-N′-nitro-N-nitrosoguanidine	$C_3H_7O_3N_5$	3.44	+	9
4-Nitroquinoline-1-oxide	$C_9H_6O_3N_2$	3.50	+	9
N-Methyl-N′-nitro-N-nitrosoguanidine	$C_2H_5O_3N_5$	3.73	+	9

[a] Carcinogenicity is indicated as follows: + = carcinogenic, 0 = non-carcinogenic, − = no data available.
In most cases chemicals were designated as carcinogenic or non-carcinogenic according to the compilations of McCann and Ames[4,9,10] or Meselson and Russell[3] and subject to their criteria. In the remaining cases carcinogenicity is based on experimental evidence that these are the active metabolites of known carcinogens. References are given to indicate the source of classification data.

sufficient for carcinogenicity. Nonetheless, we feel that the simple classification of all compounds with $Z^* < 3.2$ as potential carcinogens is not sufficiently discriminating to qualify as a predictive system since this range of Z^* values includes just as many and probably more non-carcinogens than carcinogens.

Carcinogenic potency. Prediction of carcinogenic potency is not a necessary condition for prediction of carcinogenicity, nor do Veljkovic and Lalovic make such a claim for their method. Nonetheless, a truly satisfying theory would be able to explain the order of magnitude differences in potency which are exhibited by various chemical carcino-

gens. In general, data on relative carcinogenic potencies is not easy to obtain because animal tests have not been designed to yield such information, and results may vary considerably with experimental method. However, Russel and Meselson[3] have recently compiled sufficient data to arrange 10 chemicals in order of carcinogenic potency and it is of interest to compare them for Z* value. In terms of carcinogenic potency aflatoxin > sterigmatocystin > benzo-(a)pyrene > 1,3-propanesultone > dibenz(a, h)anthracene = 4-aminobiphenyl > β-napthylanine > benzidine > MOCA > MMS. However, in order of Z* value (a low Z* value assumed to predict greater carcinogenicity), MOCA > 4-aminobiphenyl > benzidine > β-napthylamine > dibenz(a, h)anthracene > Benzo(a)pyrene > MMS > 1,3-propanesulfone > aflatoxin > sterigmatocystin. If there is any trend here at all, it would seem to run in the direction *opposite* to that proposed by Veljkovic and Lalovic.

Another point against quasi-valence number as a predictor of carcinogenicity is its insensitivity to isomerism. Recently, however, McCann and Ames have presented a series of examples of isomers which vary greatly in carcinogenic potency[4]. Thus, quasi-valence number cannot distinguish between 2-acetylaminofluorene which is carcinogenic and 4-acetylaminofluorene which is not. In the same way there are great differences in carcinogenic potency between 2-aminoanthracene and 1-aminoanthracene, β-napthlamine and α-napthylamine and 4-aminobiphenyl and 2-aminobiphenyl, yet each pair possesses identical Z* values.

Activation. Many carcinogens are not capable of damage until activated to a more reactive form. This activation may involve an increase in carcinogenic potency of an order of magnitude or more. A theory with a substantive basis in fact should reflect this, the ultimate carcinogen appearing as much more potent than the procarcinogen. Veljkovic and Lalovic, however, report a change of not more than 10%. For instance, the active form of 2-acetyl-aminofluorene is thought to be acetylaminofluorene-N-sulfate[5], yet the Z* value for this metabolite is 3.24, in the range of non-carcinogens. Similarly, Benzo(a)pyrene has a lower Z* value than its 7,8-dihydrodiol-9,10-epoxide even though the latter is thought to be the active form[6]. Finally, cycasin is activated to its carcinogenic metabolite methylazoxymethanol by gut flora[7]. Cycasin itself is not carcinogenic as indicated by its total lack of effect in gnotobiotic mice[7]. Both cycasin and methylazoxymethanol, however, have very similar Z* values.

Conclusions. The use of average quasi-valence number does make sense because a common characteristic of all carcinogens is their electrophilic nature[8] and a small number of valence electrons in an organic molecule might in some cases tend to make that molecule electrophilic. In general, it is impossible to go beyond this simple statement in predicting the carcinogenic activity of a molecule[8], although *within a closely related chemical group* there may be structural correlates. Certainly, the inverse of such a statement, namely that all electrophilic molecules are carcinogenic, is not true. Since the Z* value is essentially a quantification of this criterion, attempts to use it for prediction of carcinogenicity ought to be viewed with disfavor, at least until the correlation can be shown to hold over a broad range of chemicals. As we have illustrated, this will be difficult to do.

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Simple theoretical criterion of chemical carcinogenicity – a refutation

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Summary. The quasi-valence number criterion for chemical carcinogenicity has been shown, through several examples, to be untenable.

The article by Veljković and Lalović, which appeared in 1977 in this journal[1], is the cause of considerable concern. It claims to predict carcinogenicity and especially noncarcinogenicity ('In the case of noncarcinogenicity the quasi-valence number is necessary and sufficient criterion.') on the basis of the easily calculated 'quasi-valence number',

$$Z^* = \sum_{i=1}^{m} N_i Z_i \Big/ \sum_{i=1}^{m} N_i,$$

where N_i is the number of atoms of the i-th type in the given molecule, Z_i is the number of valence electrons in the atom of the i-th type, and m is the number of chemical elements in the molecule; except that for halogen elements $Z = 1$ instead of 7. This publication may have aroused high hopes among many of the readers of this journal for a meaningful reduction in the expense of establishing the hazard or safety of the large number of organic compounds