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Mercury isotope effects in the environmental chemistry and biochemistry of mercury-containing compounds

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Abstract. Mercury isotope effects in environmentally significant chemical and biochemical reactions are considered. The recently discovered anomaly for the ^{199}Hg and ^{201}Hg magnetic nuclei proves that both chemical and biochemical transformations of mercury compounds (outside and inside living organisms) follow radical spin-selective mechanisms. The regularities of isotope fractionation in natural photochemical processes and in living tissues are discussed. The bibliography includes 22 references.

I. Introduction

In recent years, the biogeochemical cycle of mercury and mercury compounds in the environment has become the subject of keen attention of researchers. A significant reason is realization of the fact that mercury is among the most hazardous environmental toxicants.[1,2] Being a global pollutant, mercury is encountered everywhere including the atmosphere (here its lifetime is 0.8 years), soil, ocean deposits, natural ice, natural water and living organisms, both plants and animals. Mercury occurs as different chemical species: Hg^0 atomic vapour, mono- and divalent mercury salts; methylation of the latter gives the most toxic organic compounds combined by the common name, monomethylmercury CH_3HgX (X = Cl, Br, OH, CH_3 *etc*.). The mechanisms of mercury distribution among chemical compounds, phase states and geographical ranges and its routes in food chains have become especially significant for ecology.

The principal goals of the environmental chemistry and biochemistry of mercury include elucidating the mechanisms of reversible and irreversible chemical transformations of mercury compounds, the mechanisms of interconversions between hazardous and non-toxic forms, and the relationship between purely chemical transformations (outside living organisms) and biochemical reactions (in living organisms) in the biogeochemical cycle of mercury. The latter reactions complete the most hazardous, for the living world, part of the toxic route of the mercury cycle.

In solving this problem, a prominent place belongs to isotopes; mercury isotopes and isotope effects provide a key for the understanding of the mechanisms of chemical and biochemical transformations of mercury compounds. This review is devoted to this subject.

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II. Three isotope effects

The isotope effect, *i.e*., dependence of the rate of a chemical or biochemical reaction on the nuclear composition of the reactants, is a kinetic phenomenon resulting in the fractionation of nuclei, *i.e*., their unequal proportions in the reactants and products. Three isotope effects are known:

(i) nuclear-mass effect: fractionation of nuclei is caused by the difference between their masses;

(ii) nuclear-volume effect in which the difference between the volumes (more precisely, between the radii) of the nuclei is detected;

(iii) nuclear magnetic effect where nuclear spins and magnetic moments are responsible for isotope separation.

The contribution of any of these effects to isotope fractionation is determined by the universal parameter α, single-stage separation factor, which is predetermined by the ratio of the rate constants for the reactions of molecules containing different isotope nuclei. The isotope composition of the reactant (or product) molecules and its variation during the reaction are measured experimentally; usually this is determined with respect to an isotope standard with exactly known isotope composition and is expressed as δ. Thus for ^{13}C carbon

$$\delta^{13}\mathrm{C} = \left(\frac{R_x}{R_{\mathrm{st}}} - 1\right) \times 10^3, \qquad (1)$$

where

$$R_x = \frac{[^{13}C]}{[^{12}C]}$$

is the content of ^{13}C with respect to ^{12}C in the given substance, R_{st} is the same for the standard; δ is expressed in per milles (0.1%). The relationship between δ and α is non-linear; derivation of the relationship equations was reported.[3,4]

1. Nuclear-mass isotope effect

The nuclear-mass isotope effect is the classical, long- and well-known effect having an energy nature and caused by difference between the vibrational energy of molecules with heavy and light nuclei. The former have lower energy and react more slowly than the latter; therefore, the sign of the classical effect is always positive: as a result of this effect, heavy nuclei always remain in the reactants, while light nuclei pass to the products. The effect does not depend on the chemical reaction mechanism; the parameter α is high (in some cases, up to hundreds of per cent) only for hydrogen, while for most of other elements the α values are low, about tenths of per cent, or for heavy elements, even hundredths and thousandths of per cent. However, the nuclear-mass isotope effect can be reliably detected and measured; it is widely used in all fields ranging from chemistry to archeology.

2. Nuclear-volume isotope effect

Bigeleisen[5] was the first to state the idea that in the isotope fractionation of heavy elements where the mass-dependent effects are minor, not only mass, but also the volume (more precisely the radius) of the nucleus may prove significant. The nuclear-volume isotope effect is of purely energetic origin and its magnitude is obvious from the following considerations. The potential of an electron in the centrally symmetric Coulomb field of a point nucleus equals

$$\varphi_0 = \frac{ze}{r}, \tag{2}$$

where z is the nuclear charge, e is the electron charge, r is the electron–nucleus distance. For a nucleus of radius R, the potential has the form

$$\varphi = ze\left(\frac{3}{2R}\right) - \left(\frac{r^2}{2R^3}\right). \tag{3}$$

The potential difference gives rise to a shift of the energy levels of s electrons the density of which on the nucleus differs from zero; the shift is proportional to $(R/a)^2$, where a is the Bohr orbital radius ($a = 0.525$ Å). Even for the 1s electron closest to the nucleus, this correction for the hydrogen atom is

$$\left(\frac{R}{a}\right)^2 \approx \left(\frac{0.9 \times 10^{-13}}{0.5 \times 10^{-8}}\right)^2 \approx 4 \times 10^{-10}.$$

For a large ^{196}Hg nucleus, the correction is larger

$$\left(\frac{R}{a}\right)^2 \approx \left(\frac{5.44 \times 10^{-13}}{0.5 \times 10^{-18}}\right)^2 \approx 10^{-8},$$

and increases approximately in proportion to the square of the nuclear radius.

Presumably, for p, d and f electrons whose density on the nucleus is equal to zero and for high-lying (especially valence) s electrons, these corrections may be totally ignored. However, due to the intraatomic electron exchange interaction and spin polarization, even outer (remote) electrons can, with a low probability, get on the nucleus; therefore, there are no grounds for neglecting the nuclear-volume effect for valence electrons (and, hence, for isotope chemistry); quantitative estimates and criteria of this effect are needed. Note that both the nuclear-mass and nuclear-volume effects have energy nature, but the former is determined by the vibrational energy and the latter by the energy of electrons.

The nuclear-volume effect should depend on the number of neutrons in the nucleus because the isotope nuclei being different in the mass differ also in the volume. This may depend on the number of electrons in the atom and on the atom valence state; for example, for Hg^0 atom with two valence s electrons, the effect is expected to be more pronounced than for Hg^{2+} devoid of these electrons. The effect is expected to be almost independent of the phase state of the reactants but depending on whether the atom is free or involved in a chemical bond.

The nuclear-volume isotope effect is detected rather reliably by high-resolution atomic-adsorption spectroscopy: this effect induces shifts of spectral lines, the magnitude of the shifts is about 10^{-6} of the line frequency.[6] Is the contribution of this effect to isotope fractionation significant? The answer to this question is still ambiguous (see below). Knyazev and Myasoedov[7] calculated the isotope shifts in the electronic spectra of atoms and ions and estimated the contribution of the nuclear-volume effect to the fractionation of iron, ruthenium and osmium isotopes; the isotope shifts for the extreme members of the isotope series of these elements (*i.e.*, the maximum shifts) were 0.04‰, 0.3% and 1.3‰, respectively; of course, these are very low values. Detailed calculations of the isotope shifts caused by the nuclear-volume effect for mercury and thallium were reported by Schauble.[6]

Determination of the corrections for isotope fractionation taking into account the difference in the sizes of isotope nuclei requires the use of advanced *ab initio* quantum chemical calculations. It is necessary to have exact knowledge about the electron density distribution near the nucleus, and therefore, pseudopotential methods thoroughly developed for valence electrons are inapplicable. The relativistic corrections should be precisely calculated, as the electron velocities near the nucleus in its strong Coulomb field approach the velocity of light. It is also necessary to accurately simulate the charge distribution throughout the nucleus bulk (usually this distribution is represented by the Gaussian function) and to introduce an exponential parameter that would reproduce the mean-square nuclear radii (these are known from nuclear physics; they have been measured from the nuclear-volume shifts of muon energies). Then, having calculated the corrections for one nucleus, it is necessary to find the calibration coefficients in order to determine the corrections for other isotope nuclei; calculation details were reported.[6]

For mercury, both the nuclear-mass and nuclear-volume effects were calculated; for different chemical forms [Hg^0, $HgCl_2$, $HgCl_4^{2-}$, $Hg(H_2O)_6^{2+}$, $Hg(CH_3)_2$ and so on], the values of both isotope effects were of the same order of magnitude. Note that these calculations refer to thermodynamic isotope effects, which determine the equilibrium

distribution of isotopes in natural ecosystems. We are, however, interested in the kinetic effects as a key to the understanding of reaction mechanisms.

3. Nuclear-magnetic isotope effect

Unlike the classical nuclear-mass effect, the nuclear-magnetic effect is formed by the angular momentum (full spin) of electrons and magnetic nuclei. It is manifested in the reactions of paramagnetic species (atoms, radicals, radical ions, carbenes, high-spin molecules) and follows from the fundamental and universal electron spin conservation principle: reactions are allowed only for those states of the reactants the spin of which is equal to the spin of the products; reactions are strictly forbidden if this equality does not hold.

The reactant spin can be changed, *i.e.*, the prohibition can be removed, only by magnetic interaction; the nuclear-magnetic (or simply magnetic) isotope effect (MIE) arises when this prohibition is removed as a result of magnetic interaction of the unpaired electron with the magnetic nucleus. In other words, when a paramagnetic reactant has a magnetic nucleus, the reaction is switched from the spin-forbidden channel to a spin-allowed channel. This is the nuclear-spin control of chemical reactions — a unique phenomenon where the reaction is controlled by the magnetic moment of reactant nuclei rather than by their masses (MIE is described in detail in the literature.[4,8]).

The appearance of a MIE in the reaction proves unambiguously that this reaction is spin-selective giving rise to spin-bearing paramagnetic species with open electron shells. Unlike the nuclear-mass effect, which exists in all reactions and at any mechanisms, MIE is indicative of exactly a spin-selective reaction. In this respect, its meaning is absolute: this is the only isotope effect that actually detects the mechanism; below it is used to analyze mercury-containing compounds.

Mercury comprises seven stable isotopes of which ^{198}Hg (10.1), ^{199}Hg (17.0), ^{200}Hg (23.1), ^{201}Hg (13.2), ^{202}Hg (26.65) and ^{204}Hg (6.85) are significant (the value in parentheses is the natural abundance in %). Of these, the odd isotopes ^{199}Hg and ^{201}Hg have spins and magnetic moments (1/2 and 0.5059, 3/2 and −0.5602, respectively), while all even isotopes are spinless and non-magnetic.

The spin conversion in radical and radical-ion pairs involving mercury-centred radicals is controlled by hyperfine (magnetic) coupling of the unpaired electron with the mercury magnetic nucleus. The coupling constants are known only for few radicals (expressed in Gauss):

CH_3Hg	HgH	HgCN	HgF	$(CH_3)_2Hg^-$
1758	2450	5700	7920	2674

These constants refer to ^{199}Hg but can easily be converted to ^{201}Hg. For X_2Hg^+ only one of the components of the hyperfine coupling tensor is known but it differs little from the same values for neutral radicals and radical anions.[9,10]

III. Isotope effects in photochemical reactions

1. Photolysis of benzylmercury chloride

Photolysis of $PhCH_2HgCl$ in benzene is accompanied by fractionation of ^{13}C isotope: unreacted benzyl chloride is enriched in ^{13}C by 0.25‰.† This result is in line with the chemically induced nuclear polarization: a triplet radical pair ($PhCH_2^{\bullet}$ $^{\bullet}HgCl$) is a spin-selective nanoreactor in which the presence of magnetic ^{13}C nuclei accelerates the triplet–singlet conversion and stimulates recombination of the pair to give initial benzyl chloride enriched in ^{13}C. The radicals $PhCH_2^{\bullet}$ carry the excess of ^{12}C into dibenzyl formed upon their recombination.[11]

The observed fractionation of the carbon nuclei cannot be attributed to the MIE alone, because the classical mass-dependent effect has the same sign and should also result in accumulation of heavier nuclei in the starting compound. Unambiguous conclusion about the contributions of both effects was drawn only from analysis of the fractionation of mercury isotopes in the photolysis of another organomercury compound, monomethylmercury CH_3HgX;[12] this compound is often used by geochemists-environmentalists. The composition of the compound is variable, the substituent X is represented by Cl, Br, OH and CH_3 in different ratios. An advantage of this compound is that its chemical and photochemical behaviour simulates the natural reactions of mercury compounds with a mercury–carbon covalent bond.

2. Photolysis of monomethylmercury and reduction of mercury salts

Aqueous solutions of monomethylmercury (MMM) containing dissolved organic matter [also having an uncertain composition and called Dissolved Organic Carbon (DOC) by geochemists] were exposed to sunlight (July, August, 2006) in quartz vessels (at the latitude of Michigan, USA) and purged with purified air. The atomic mercury Hg^0 formed in the photolysis was removed by air: the photo-induced synthesis of Hg^0 was isotope-dependent and was accompanied by fractionation of mercury isotopes. Their contents in the reactor (*i.e.*, in the unreacted MMM residue) were measured by high-precision inductively coupled plasma mass spectrometry and the MMM content in the residue (*i.e.*, chemical conversion of MMM) was determined by atomic-absorption spectroscopy.[12]

The isotope shifts [see Eqn (1)] for isotopes ^{199}Hg, ^{200}Hg, ^{201}Hg, ^{202}Hg and ^{204}Hg were measured with respect to the lightest ^{198}Hg isotope. In all cases, MMM with light isotopes reacted faster, *i.e.*, the initial solution was enriched in heavy isotopes; the heavier the isotope, the larger its amount remained. The enrichment also became more pronounced with the increase in the conversion: the less MMM remained in the solution, the more heavier isotopes it contained.

These results are usual for the practice of isotope chemistry but the difference between the behaviour of even and odd mercury isotopes is unexpected. The isotope shifts (δHg) for the ^{198}Hg, ^{200}Hg, ^{202}Hg and ^{204}Hg nuclei depend exactly on the mass, which points unambiguously to the fact that the fractionation of spinless non-magnetic nuclei strictly obeys the classical mass-dependent isotope effect. (Note on the spot that the nuclear volumes are different but this does not affect their fractionation, *i.e.*, no nuclear-volume isotope effect is here involved.)

A clear-cut abnormal behaviour was found for odd isotopes with spin magnetic nuclei, ^{199}Hg and ^{201}Hg: their content in the residue exceeded the value expected for the classical isotope effect (Fig. 1). The deviation is measured as the difference between the observed isotope shift $(\delta^{199}\mathrm{Hg})_{\mathrm{obs}}$ and the expected mass-dependent shift $(\delta^{199}\mathrm{Hg})_{\mathrm{mass}}$

$$\Delta^{199}\mathrm{Hg} = (\delta^{199}\mathrm{Hg})_{\mathrm{obs}} - (\delta^{199}\mathrm{Hg})_{\mathrm{mass}}\,. \qquad (4)$$

† Unpublished data of the author.

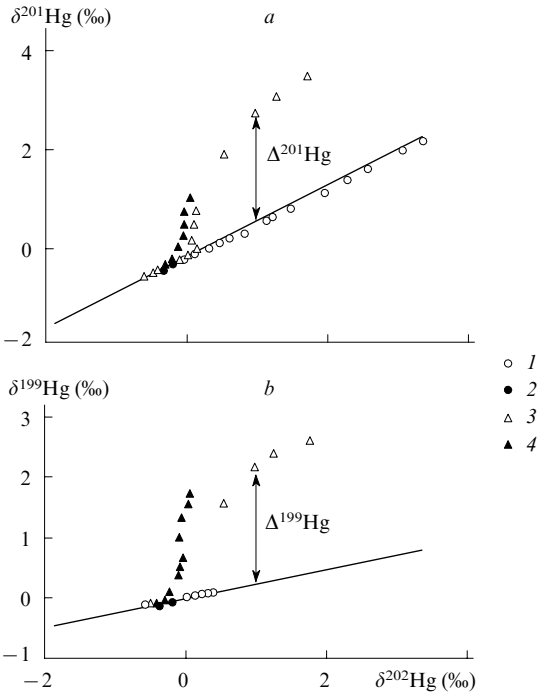


Figure 1. Relationship between the isotope shifts of the magnetic nuclei ^{201}Hg (*a*) and ^{199}Hg (*b*) and the non-magnetic nucleus ^{202}Hg. The lines correspond to the classical isotope effect, deviations from them for ^{201}Hg and ^{199}Hg determine the size of magnetic-isotope anomaly.
(*1*, *2*) Isotopic composition for the dark biogenic and abiogenic reduction of Hg^{2+}; (*3*, *4*) photoreduction of Hg^{2+} and photolysis of MMM, respectively.

The same expression for Δ^{201}Hg has the form

$$\Delta^{201}\mathrm{Hg} = (\delta^{201}\mathrm{Hg})_{\mathrm{obs}} - (\delta^{201}\mathrm{Hg})_{\mathrm{mass}}\,. \qquad (5)$$

The plots shown in Fig. 1 demonstrate that the Δ values for ^{199}Hg and ^{201}Hg are great and markedly exceed the values predicted by classical mass-dependent effect (straight lines). (Recall that the isotope shifts were measured relative to isotope ^{198}Hg).

It is significant that Δ^{199}Hg and Δ^{201}Hg are nearly the same and their ratio is close to the ratio of magnetic moments of the ^{201}Hg and ^{199}Hg nuclei ($\sim$1.1). This indicates that magnetic isotope effect makes a considerable contribution to isotope separation together with the classical isotope effect: it returns the magnetic nuclei ^{199}Hg and ^{201}Hg to the starting reactant much more efficiently than the nuclear-mass effect. (Note also that the nuclear-volume factors for ^{199}Hg and ^{201}Hg differ by a factor of almost three; however, these are not manifested in the isotope separation of these nuclei, which is the same and does not depend on the nuclear volume.)

The isotope shifts δHg and ΔHg prove unambiguously that two of the three known isotope effects, namely, the classical mass-dependent and nuclear-magnetic effects are responsible for fractionation of mercury isotopes in the photolysis of MMM. This is even more evident from the dependences of the absolute abundances of mercury isotopes in the remaining MMM on the degree of its conversion, *i.e.*, on the fraction of the remaining MMM. The $^{*}S_f$ value defined by the relation

$$^{*}S_f = \left(\frac{^{*}\mathrm{Hg}}{^{198}\mathrm{Hg}}\right)_f \left(\frac{^{*}\mathrm{Hg}}{^{198}\mathrm{Hg}}\right)_0^{-1}, \qquad (6)$$

characterizes the isotope composition of the remaining compound for its fraction being *f* with respect to the initial isotope composition before the reaction where $f = 1$ (the asterisk marks any isotope except for ^{198}Hg relative to which the isotope composition is measured).

These dependences for ^{199}Hg, ^{200}Hg, ^{201}Hg and ^{202}Hg isotopes are shown in Fig. 2. It can be seen that accumulation of odd magnetic isotopes in initial MMM increases with conversion much more rapidly than that of even non-magnetic isotopes, *i.e.*, the nuclear-magnetic effect sorts the nuclei much more efficiently than the nuclear-mass effect.

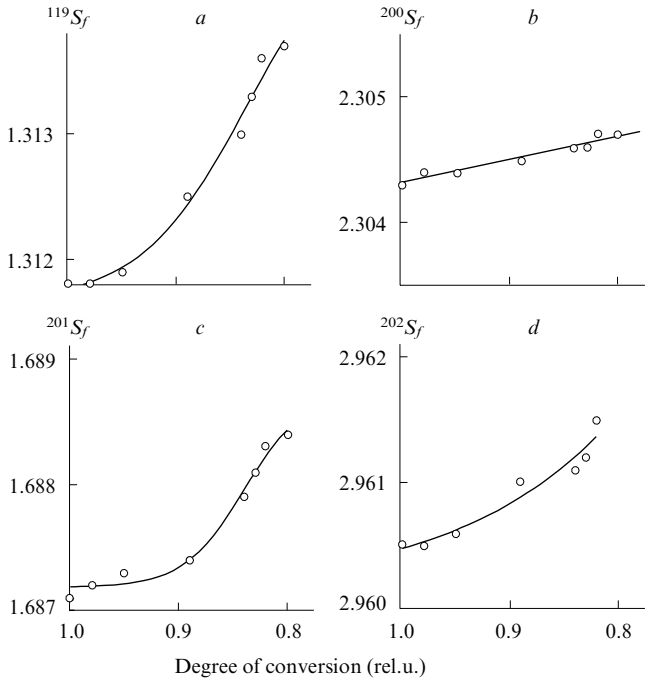


Figure 2. Isotopic composition for ^{199}Hg (*a*), ^{200}Hg (*b*), ^{201}Hg (*c*) and ^{202}Hg (*d*) in MMM *vs.* chemical conversion degree.

Apart from MMM photolysis, phororeduction of mercury(II) was studied [12] using the same procedure as for MMM photodecomposition. This reaction follows the same trends as MMM photodecomposition: the isotope abundances in the initial reactant increase as its photodecomposition goes on; enrichment in the odd isotopes ^{199}Hg and ^{201}Hg is more pronounced than in the even ones; fractionation is also provided by two isotope effects, the classical nuclear-mass effect and nuclear-magnetic one. We converted the δHg values reported in the study cited [12] into the α values, which are isotope fractionation factors (Fig. 3). (Note that, as in the case of MMM, these are purely isotope effects involved in the reactions, as other possible sources of fractionation were excluded in control experiments.) The α values for the even isotopes (recall once again that both δHg and α were measured with respect to the isotope ^{198}Hg) follow a strictly linear dependence on the mass, and only for the magnetic ^{201}Hg nucleus, does the measured $^{201}\alpha$ value exceed considerably the $^{201}\alpha_{\mathrm{mass}}$ value expected for the mass-dependent effect (see Fig. 3).

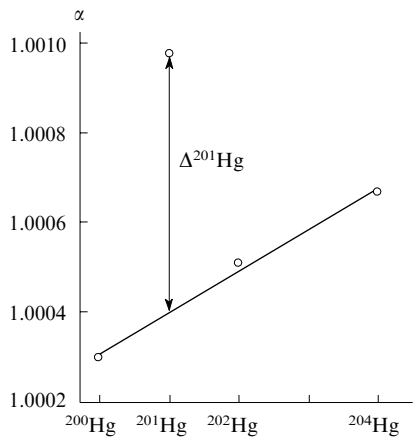


Figure 3. Isotopic fractionation factors for mercury isotopes in MMM photolysis.
For even (non-magnetic) isotopes, α values correspond to the classical nuclear-mass isotope effect and depend linearly on the nuclear mass; for the magnetic isotope ^{201}Hg, α is abnormally high.

For coexistence of two isotope effects, the total (summarized) isotope fractionation factor is known to be equal to the product of the factors;[4] for $^{201}\alpha$ this product is given by

$$^{201}\alpha = {}^{201}\alpha_{\text{mass}}\,{}^{201}\alpha_{\text{magn}}. \qquad (7)$$

Now we can quantitatively separate the contributions of the two effects to isotope selection. By substituting the values (see Fig. 3)

$$^{201}\alpha_{\text{mass}} = 1.0004,$$

$$^{201}\alpha = 1.0010,$$

into Eqn (7), we get

$$^{201}\alpha_{\text{magn}} = 1.0006.$$

It is clear that the magnetic isotope effect surpasses the classical nuclear-mass effect. The absolute measure of this surpass is given by the ratio

$$\frac{1.0006 - 1}{1.0004 - 1} = 1.5,$$

i.e., the magnetic effect sorts the ^{201}Hg nuclei 1.5 times more efficiently than the classical nuclear-mass effect.

Now we consider the principal aspect: in which reactions the isotope sorting takes place. The significant fact that the crucial role in the isotope fractionation of odd nuclei belongs to MIE implies that both reactions, MMM photodecomposition and Hg^{2+} photoreduction, involve radical or radical-ion intermediates. The accumulation of magnetic nuclei in the starting reactants can be reasonably described in terms of the following reaction schemes:

$$CH_3HgX \xrightleftharpoons[^{199}\text{Hg},\,^{201}\text{Hg}]{h\nu} (\dot{C}H_3\,\dot{H}gX)^{T} \longrightarrow \text{products (including } Hg^0),$$

$$Hg^{2+} + Y \xrightleftharpoons[^{199}\text{Hg},\,^{201}\text{Hg}]{h\nu} (\overset{+}{H}\dot{g}\;\dot{\overset{+}{Y}})^{T} \longrightarrow \text{products},$$

where Y is a dissolved organic compound (DOC). In the former case, the triplet radical pair acts as the spin-selective nanoreactor, and in the latter case, this is the triplet radical ion pair. Both pairs experience fast triplet–singlet spin conversion if the mercury-centred radicals contain magnetic mercury nuclei. In the singlet state, both pairs recover the starting reactants (upon radical recombination in the former case and charge disproportionation in the latter case), thus bringing excess magnetic nuclei into them. For the pairs with non-magnetic nuclei, the triplet–singlet spin conversion lags behind, these pairs being preferably converted into the products (finally into Hg^0) thus bringing excess non-magnetic nuclei into them. The chemical mechanisms of photodecomposition and photoreduction of mercury compounds formulated on the basis of isotope effects and isotope fractionation play a key role in the chain of chemical transformations of mercury in natural ecosystems.

There is yet another evidence in favour of the presented mechanisms. The isotope distribution of mercury in the dark reactions of Hg^{2+} with DOC has been studied.[12] In this case, only the nuclear-mass effect was observed, and the magnetic nuclei ^{199}Hg and ^{201}Hg were not distinguished from the common trend. In other words, in the dark reactions no MIE is manifested, as was to be expected because in the dark reactions, the spin-selective nanoreactor (radical pair) arises in the singlet state (it inherits the zero spin of the reactants) in which the nuclear-magnetic fractionation is low and is inferior to mass-dependent fractionation.[3]

3. Quantitative measure of isotope effects

The nuclei ^{199}Hg and ^{201}Hg are test objects suitable for quantitative comparison of the nuclear-volume and nuclear-magnetic isotope effects. The nuclear-volume factor for ^{199}Hg is almost three times as high as this factor for ^{201}Hg; a calculation predicts[13] that this difference should result in Δ^{199}Hg being twice as high as Δ^{201}Hg, *i.e.*, the fractionation of these isotopes should differ by a factor of two. Conversely, the nuclear-magnetic effect is determined by the spin conversion rate and is expected to be nearly equal for both nuclei because their magnetic moments differ by only 10%.

Which of these effects predominates and to what extent? An answer to this question can be gained by comparing Δ^{199}Hg and Δ^{201}Hg: they are nearly equal. However, the strict answer can be obtained only by comparing the single-stage fractionation factors, $^{199}\alpha$ and $^{201}\alpha$, which are fundamental characteristics of any isotope effect. The parameter α is the ratio of the reaction rates of the molecules incorporating different isotopes. However, the α values are not linearly related to ΔHg but through a sequence of equations, which will now be derived and the final relation will be used to determine α from ΔHg.

The relationship between α and ΔHg will be derived using the ^{199}Hg abundance (determined experimentally) as an example. The starting equation that follows from Eqn (1) has the form

$$\Delta^{199}\text{Hg} = \left[\frac{^{199}\text{Hg}/^{198}\text{Hg}}{\left(^{199}\text{Hg}/^{198}\text{Hg}\right)_{\text{st}}}\right] \times 10^3, \qquad (8)$$

where Δ^{199}Hg is measured experimentally (in ‰);

$$\frac{^{199}\text{Hg}}{^{198}\text{Hg}} \text{ and } \left(\frac{^{199}\text{Hg}}{^{198}\text{Hg}}\right)_{\text{st}}$$

are the ratios of the isotope abundances in the tested substance (*e.g.*, in MMM) and in the standard. Now we introduce a physically clear quantity

$$^{199}S_f = \left(\frac{^{199}\mathrm{Hg}}{^{198}\mathrm{Hg}}\right)_f \left(\frac{^{199}\mathrm{Hg}}{^{198}\mathrm{Hg}}\right)_0^{-1}, \quad (9)$$

which determines the ratio of the isotope composition of the substance in its residue with respect to the initial isotope composition. Combining Eqns (8) and (9) gives a simple expression for $^{199}S_f$

$$^{199}S_f = \frac{\left(10^{-3} \times \Delta^{199}\mathrm{Hg} + 1\right)}{\left(10^{-3} \times \Delta_0{}^{199}\mathrm{Hg} + 1\right)}. \quad (10)$$

Here, Δ^{199}Hg in the numerator refers to the remaining substance when its content is f; $\Delta_0{}^{199}$Hg in the denominator refers to the starting compound, *i.e.*, to $f = 1$.

The $^{199}S_f$ value is related to the chemical conversion of the analyzed compound by the equation

$$\ln {}^{199}S_f = \frac{1 - {}^{199}\alpha}{{}^{199}\alpha} \ln f, \quad (11)$$

derivation of which has been reported.[4,8] This equation is applicable to any isotope effect and has been validated reliably for various isotopes and many reactions.

By combining Eqns (10) and (11) taking into account the fact that $\ln(1+x) \approx x$ for $x \ll 1$, we obtain the equation relating α and ΔHg

$$^{199}\alpha = \frac{\ln f}{\ln f + 10^{-3}\left(\Delta^{199}\mathrm{Hg} - \Delta_0^{199}\mathrm{Hg}\right)}. \quad (12)$$

Similarly,

$$^{201}\alpha = \frac{\ln f}{\ln f + 10^{-3}\left(\Delta^{201}\mathrm{Hg} - \Delta_0^{201}\mathrm{Hg}\right)}. \quad (13)$$

Note that in these equations all values are dimensionless.

Using Eqn (12) and (13), let us process the data of one MMM photolysis experiment from the publication[12] (see Ref. 12, Supplementary Materials, Table 1S). For $f = 0.8$, Δ^{199}Hg = 1.69‰, $\Delta_0{}^{199}$Hg = 0.02‰, Δ^{201}Hg = 1.22‰, $\Delta_0{}^{201}$Hg = 0.01‰. From this, we have $^{199}\alpha = 1.0075$, $^{201}\alpha = 1.0054$, which attests in favour of MIE as the predominant factor in isotope sorting; the contribution of the nuclear-volume effect is insignificant.

For Hg^{2+} photoreduction (see Ref. 12, Supplementary Materials, Table 2S), the fractionation factors are lower: $^{199}\alpha = 1.00093$, $^{201}\alpha = 1.00094$. The fact that they are nearly equal is also indicative of the predominant contribution of MIE to the sorting of nuclei; however, the low α values for this reaction are unexplainable. The point is that the hyperfine coupling constants for the mercury nuclei in neutral mercury-centred radicals and in the radical cations with Hg^+ differ insignificantly; therefore, the α values for both reactions are expected to be similar. Low α values for the photoreduction may imply the existence of a singlet reaction channel along with the triplet one; due to the inversion of the MIE sign, the singlet channel may decrease the efficiency of fractionation of magnetic isotopes along the triplet channel.

4. Photolysis of dibenzylmercury: sign inversion of the magnetic isotope effect

An unambiguous proof for the fact that fractionation of the ^{199}Hg (and, hence, of ^{201}Hg) magnetic isotope is due to the MIE was gained in a study of the photolysis of di(*p*-trifluoromethylbenzyl)mercury (**1**) in heptane, isopropyl alcohol and chloroform.[14] This reaction gives dibenzyl and mercury metal. The ^{199}Hg abundance in compound **1** was measured by NMR as the integral intensity of the $^{13}C-{}^{199}Hg$ satellites in the spectrum of the $^{13}CH_2$ group with natural abundance of both isotopes, ^{13}C and ^{199}Hg.

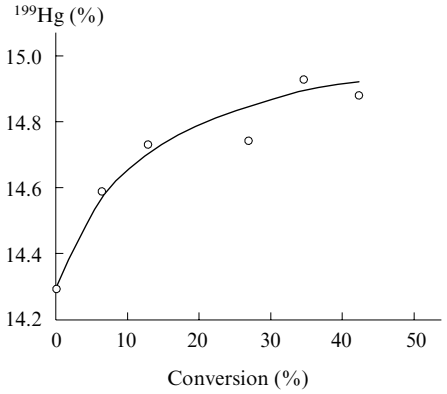


Figure 4. ^{199}Hg abundance in compound **1** *vs.* its chemical conversion.

The plot in Fig. 4 shows how the absolute abundance of ^{199}Hg in the remaining compound **1** increases in the course of photolysis; when the conversion of **1** is ~50%, the gain of ^{199}Hg in its residue exceeds 4%. The composition of the products and the isotope fractionation are described by Scheme 1.

Scheme 1

$$(p\text{-}CF_3C_6H_4CH_2)_2Hg \underset{^{199}Hg}{\overset{h\nu}{\rightleftharpoons}} (CF_3C_6H_4\dot{C}H_2\ \dot{H}gCH_2C_6H_4CF_3)^T \xrightarrow{^{200}Hg}$$

$$\longrightarrow CF_3C_6H_4\dot{C}H + \dot{H}gCH_2C_6H_4CF_3 \longrightarrow (CF_3C_6H_4CH_2)_2 + Hg.$$

The isotope sorting takes place in the triplet radical pair formed upon C–Hg bond dissociation in the initial triplet excited molecule. The pairs with the ^{199}Hg magnetic nucleus rapidly pass from the triplet to singlet state and recombine, thus returning the magnetic nuclei into the starting molecule **1**. The triplet–singlet conversion of pairs with non-magnetic nucleus (*e.g.*, ^{200}Hg) lags behind and these pairs dissociate into radicals. The subsequent reactions of these radicals give the products, substituted dibenzyl and mercury metal (see Scheme 1).

The inversion of the isotope effect sign provides an unambiguous proof for the fact that the nuclear-volume isotope effect is insignificant. As shown by Buchachenko *et al.*,[15] molecule **1** is enriched in ^{199}Hg isotope under the conditions where the suspension (emulsion) of colloid mercury has been precipitated and photolysis occurs for transparent solutions of **1**. However, on continuous photolysis with irradiation of solutions containing colloid mercury, molecule **1** is depleted in the ^{199}Hg isotope. The inversion of the isotope effect sign means that under these conditions, decomposition of molecule **1** takes place from

the singlet excited state giving rise to a singlet radical pair in which the direction of spin conversion and, hence, the isotope selection change the sign.

The most probable reason for inversion is photolysis of compound **1** adsorbed on highly dispersed mercury. However, the question why an electronically excited molecule in solution decomposes from the triplet state and an adsorbed molecule, from the singlet state, cannot be answered unambiguously. The following explanation is most probable.

In the adsorbed state, binding of the mercury atom of a molecule to a surface atom of mercury metal results in additional 'freezing' of the molecular orbital moment (as occurs in strong electric fields of usual chemical bonds). This 'freezing' weakens the inner spin – orbit coupling in the adsorbed molecule and retards intramolecular singlet – triplet conversion. As a result, the competing decomposition of the singlet molecule at the C—Hg bond with generation of a singlet radical pair predominates. This competition results in the sign inversion of the magnetic isotope effect. Unambiguously known is only the fact that the sign inversion is a direct consequence of the MIE; neither the nuclear-mass nor the nuclear-volume isotope effect is accompanied by inversion.

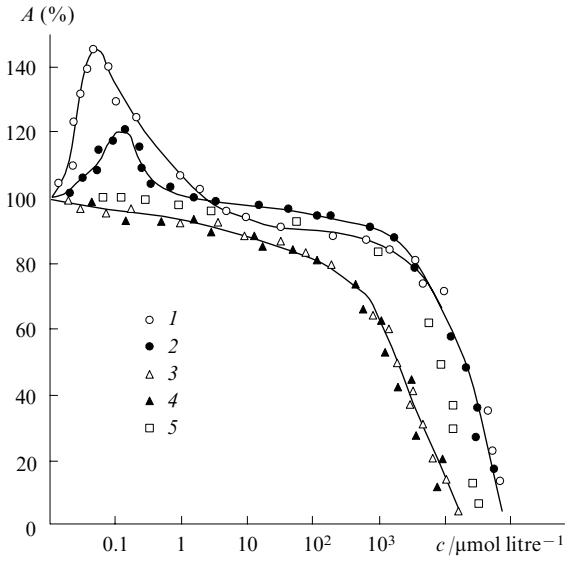


Figure 5. Creatine kinase relative activity [A (%)] *vs.* concentration of CH_3HgCl (c) with different mercury isotopes.
(*1*) ^{199}Hg, (*2*) ^{201}Hg, (*3*) ^{200}Hg, (*4*) ^{202}Hg, (*5*) natural abundance.

IV. Mercury isotope effects in natural biosystems

1. Isotope effect in the synthesis of adenosine triphosphate by creatine kinase

Creatine kinase (CK) is a phosphorylating enzyme producing adenosine triphosphate (ATP), which is the main energy carrier in any living organism. The enzyme operates reversibly, *i.e.*, either synthesizes ATP from adenosine diphosphate (ADP) and creatine phosphate (CP) or performs the back reaction, CP synthesis from ATP.

Methylmercury chloride is one of the strongest CK inhibitors, the key point in the inhibitory effect of CH_3HgCl being suppression of phosphorylation.[16]

To elucidate the nature of the poisoning action of CH_3HgCl on CK, the reaction of the latter with pure isotope forms of methylmercury chloride: $CH_3{}^{199}HgCl$, $CH_3{}^{200}HgCl$, $CH_3{}^{201}HgCl$ and $CH_3{}^{202}HgCl$, was studied.[17] An established dependence of the poisoning effect on the isotope composition of CH_3HgCl was a remarkable and absolutely unexpected result of this study. Figure 5 shows these dependences for different isotopes.

First, enzyme activation is observed at low methylmercury chloride concentrations but only for isotopes with magnetic nuclei, while spinless nuclei ^{200}Hg and ^{202}Hg do not show this effect. Methylmercury chloride with the natural abundance of isotopes did not show this effect either; in this case, the content of magnetic isotopes does not exceed 30%, *i.e.*, the contribution of magnetic CH_3HgCl molecules is insufficient to be manifested in enzyme activation.

Second, at high inhibitor concentrations, the CK activity is considerably suppressed but the suppression effect is also dependent on the isotope. The CH_3HgCl concentration that suppresses the enzyme activity by 50% (c_{50}) is 42 mmol litre^{-1} for magnetic CH_3HgCl forms and 2.8 mmol litre^{-1} for non-magnetic forms. It is clear that CH_3HgCl with ^{199}Hg or ^{201}Hg nuclei is much 'safer' for CK than the molecules with spinless ^{200}Hg and ^{202}Hg nuclei: poisoning by the former occurs later and at higher concentrations than poisoning by the latter. In the case of CH_3HgCl with the natural abundance of mercury isotopes, the c_{50} value lies between these two limits and is approximately proportional to the contributions from both magnetic and non-magnetic isotopic forms (see Fig. 5).

In both the former and the latter case, the reactivities of CH_3HgCl molecules with magnetic and non-magnetic mercury nuclei are found to be different, *i.e.*, the magnetic isotope effect is manifested, but there is no difference between ^{199}Hg and ^{201}Hg or between ^{200}Hg and ^{202}Hg isotopes; in other words, there are no signs of classical nuclear-mass isotope effect. Thus, the reaction between CK and CH_3HgCl is spin-selective, being dependent on the nuclear magnetic moment, and, hence should be isotope-sorting.[17]

This assumption was verified by studying the isotope composition of CH_3HgCl incubated with CK at natural abundance of the four mercury isotopes. It was found that the major part ($\sim$80%) of CH_3HgCl incubated was extractable into acetone and the isotope composition of this fraction differed slightly from that of the starting methylmercury chloride. However, the other part (20%) of the starting chloride could not be extracted as it was chemically bound to CK. The isotope composition of this mercury-containing fraction (which was analyzed together with the protein) differed sharply from that of the starting chloride, being 15% – 17% enriched in the magnetic isotopes ^{199}Hg and ^{201}Hg and 16% – 18% depleted in the non-magnetic isotopes ^{200}Hg and ^{202}Hg (Fig. 6). The isotope ratio within the fractions remained invariable, *i.e.*, again, no signs of classical isotope effect were observed.

Note that to within the experimental error, the overall isotope balance between two CH_3HgCl fractions, one extracted with acetone and one chemically bound to the protein, is satisfactorily conserved. Both the inhibitory effect (see Fig. 5) and isotope fractionation (see Fig. 6) convincingly demonstrate the nuclear-spin selectivity of the reaction of the enzyme with CH_3HgCl, which can be followed by examining Scheme 2.

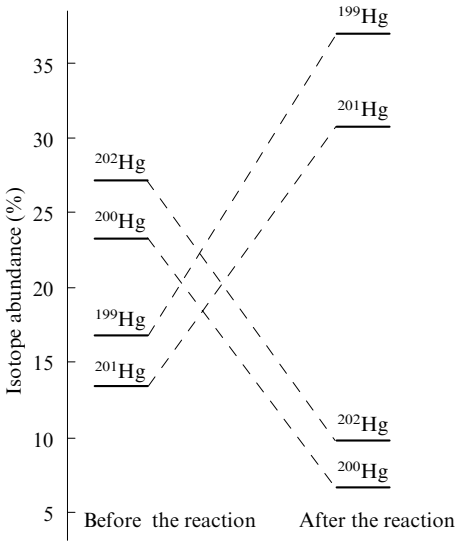


Figure 6. Isotope abundances of methylmercury chloride before the reaction and after the reaction (extracted fraction) with creatine kinase.

Scheme 2

$$CH_3HgCl + HSR \underset{}{\overset{^{200}Hg,\ ^{202}Hg}{\rightleftharpoons}} [CH_3\dot{H}g\bar{C}l\ \overset{+}{H}\dot{S}R]^S \xrightarrow{^{199}Hg,\ ^{201}Hg}$$

$$\longrightarrow [CH_3\dot{H}g\bar{C}l\ \overset{+}{H}\dot{S}R]^T \xrightarrow[-HCl]{} [CH_3\dot{H}gCl\ \dot{S}R] \longrightarrow$$

$$\longrightarrow CH_3HgSR$$

where HSR is cysteine.

The Hg–Cl bond is known to be non-polar and not susceptible to nucleophilic or electrophilic attack but prone to oxidation; in particular, CH_3HgCl actively reacts with the SH groups. A key structural unit of CK is cysteine, which keeps creatine in the catalytic site and positions it along the reaction coordinate. A nuclear-spin selectivity should be sought in the reaction of CH_3HgCl with cysteine.

The first step in Scheme 2 is generation of a radical ion pair upon electron transfer from cysteine to CH_3HgCl. Like any other thermally induced process, this reaction gives rise to a singlet pair, its partners are held by the Coulomb attraction. This primary pair is a spin-selective nanoreactor in which the hyperfine coupling of the unpaired electron in the CH_3^- $^{\bullet}$HgCl radical anion induces a singlet–triplet conversion of the pairs with ^{199}Hg and ^{201}Hg magnetic nuclei. The spin conversion of the pairs with non-magnetic ^{200}Hg and ^{202}Hg nuclei lags behind, these pairs recovering the starting reactants upon back electron transfer thus bringing there the non-magnetic isotopes.

The triplet pairs with excess ^{199}Hg and ^{201}Hg nuclei cannot recover the reactants, as the back electron transfer in them is spin-forbidden. In this case, the only chemically available channel is release of HCl and transformation into the secondary triplet pair ($CH_3Hg^{\bullet}$ $^{\bullet}$SR). In the radical $CH_3Hg^{\bullet}$, the hyperfine coupling is enormous: $\sim$1700 G for ^{199}Hg and 630 G for ^{201}Hg;[9] which corresponds to a spin conversion period of $\sim 10^{-10}$ s. Therefore, the pair rapidly becomes singlet and the partners recombine. The recombination is accompanied by two effects: first, the chemical addition of a mercury-containing radical to the protein and, second, enrichment of the protein in the magnetic isotopes ^{199}Hg and ^{201}Hg. Both are in line with experimental results.[17]

Note an important fact: methylmercury chloride does not 'interfere' with the proper enzymatic process, the phosphate group transfer, but only violates creatine kinase by distorting the structure of its catalytic sites and inhibiting the enzymatic activity; CH_3HgCl (and generally MMM) can react with the protein SH groups by this mechanism also in living organisms, which is accompanied by isotope fractionation; examples are given below.

2. Isotope effects in natural biosystems

Mercury isotope analyses in biosystems are numerous and diverse but non-systematic and absolutely uncoordinated. However, even this fragmentary knowledge shows some common trends in the chemical and biochemical fate of mercury in living systems.

Recently, Epov *et al.*[18] have developed a reliable and accurate method for the determination of the total isotopic composition of mercury in a single sample on a real-time basis by combining gas chromatography and multicollector inductively coupled plasma mass spectrometry. Using these methods, the isotopic composition of mercury in fish tissue where mercury occurs by 92%–98% as MMM, was determined. The isotope shifts (measured with respect to ^{198}Hg) for the even spinless isotopes were found to strictly correspond to the classical isotope fractionation effect; however, the magnetic isotopes, ^{199}Hg and ^{201}Hg, were present in considerable excess: Δ^{199}Hg were +2.16‰ and +2.37‰ (for two series of measurements), Δ^{201}Hg were +1.77‰ and +2.13‰. These isotope shifts were almost identical to those arising in the MMM photolysis; therefore, the authors concluded that MMM gets to the tuna fish from the environment with a 'pre-prepared' isotopic composition.

Similar measurements of mercury isotope shifts in fish (burbot) tissue from the Michigan Lake, New England Lakes (Canada) and in sea fish were reported.[19] The δ^{202}Hg was unambiguously correlated with the total mercury content in the tissues and showed a dependence on fish size, age and trophic level (position in the food chain). The abundances of heavy isotopes in the tissues increase with age. The researchers concluded that isotope shifts can be used as a measure of accumulation of mercury in tissues. The abundances of non-magnetic isotopes obeyed the classical isotope effect, whereas the abundances of magnetic isotopes ^{199}Hg and ^{201}Hg demonstrated the same abnormal behaviour as in the MMM photolysis: the Δ^{199}Hg and Δ^{201}Hg values reached 4‰–5‰ and the linear relationship between them was identical to that in the photolysis of MMM. The slope ratio of this straight line was also close to the ratio of the ^{199}Hg and ^{201}Hg magnetic moments. Thus, it was concluded that mercury in fish tissues occurs mainly (>80%) as MMM, and the variations of Δ^{199}Hg and Δ^{201}Hg in the tissues of fishes from different lakes reflect the level of habitat ecological well-being depending, in particular, on the wind diagram: mercury compounds originate from industrially developed and environmentally adverse areas.

Odom and co-workers[13] measured the isotopic composition of mercury in the peat from the Patagonia and Pemido Vella (Spain) swamps and in the moss of Tallahassee (Florida). The abundances of even isotopes corresponded to the classical isotope effect, whereas odd isotopes showed the same abnormal behaviour as in the

MMM photolysis. Moreover, the Δ^{199}Hg and Δ^{201}Hg values for all samples fitted in a single linear dependence with a slope ratio of 1.05 close to the ratio of the nuclear magnetic moments. The authors, however, concluded that fractionation of isotopes in the Spanish peat and moss is determined by the magnetic isotope effect, while in the Patagonia peat, the nuclear-volume isotope effect is responsible for ^{199}Hg and ^{201}Hg fractionation. However, the grounds for this conclusion are unreliable, as deviations in the Δ values from the straight line with a slope ratio of 1.05 for both magnetic isotopes do not go beyond the error of measurements of isotope shifts.

An extensive study of the isotopic composition of mercury in the tissues of inhabitants of Canadian lakes of three trophic levels (crustaceous, small fishes and predaceous fishes) and in bottom sediments was carried out by Jackson *et al.*[19] In the bottom sediments, no isotope anomalies were found, the isotope shifts for all nuclei corresponded to the classical mass-dependent isotope effect. In living organisms, however, the content of magnetic nuclei ^{199}Hg and ^{201}Hg substantially exceeded that to be expected of the classical effect. Moreover, it increased in the trophic series from crustaceans to predaceous fishes and also depended on whether the fishes lived in cold bottom or in warm subsurface waters. In particular, MMM extracted from lake trout had an abnormally high abundances of ^{199}Hg and ^{201}Hg. The authors concluded that isotope effects arise at the microbial level in biochemical reactions. This might be spin-selective reactions of MMM with SH groups of biomolecules similar to the reaction of CH_3HgCl with creatine kinase (see Section IV.1); unambiguous conclusions have not been drawn as yet.

The fractionation of ^{202}Hg with respect to ^{198}Hg occurred in Hg^{2+} reduction to Hg^0 during cell division of a gram-negative *E. coli* strain.[20] The fractionation factors varied in the range of 1.0013 – 1.0020 depending on temperature; Hg^{2+} was enriched in the heavy isotope, which is indicative of the mass-dependent effect. It is impossible to draw any conclusions about the reaction mechanism, since the most 'informative' isotopes, ^{199}Hg and ^{210}Hg, were not analyzed in the study.

V. Conclusion

Studies of mercury isotope effects considerably extended the knowledge in two fields — in the environmental chemistry and biochemistry and in the physics of isotope effects. The fact of coexistence of even and odd isotopes is a reliable key for the understanding of chemical reaction mechanisms. The mass-dependent effect is reliably detected for even isotopes; however, no conclusions on the reaction mechanism can be drawn from merely this observation. A considerable isotope anomaly for the odd (magnetic) isotopes unambiguously proves the radical or radical-ion mechanism of photochemical transformations both in the decomposition of organomercury compounds and in the reduction of inorganic mercury compounds. This anomaly is manifested in both photoinduced and dark reactions of biomolecules (the latter was demonstrated in relation to creatine kinase).

It was found that monomethylmercury is the major toxicant. This follows from the fact that changes in its isotopic composition during photolysis in nature (under sunlight) are very close to the isotope anomaly (for ^{199}Hg and ^{201}Hg) detected in living tissues (in particular, in fishes). However, it is still unknown whether this isotope anomaly is created in the open system and then transferred into living organisms together with methylmercury or created independently in the biochemical reactions (as in creatine kinase).

It can also be concluded from analysis of isotope effects that mercury release to the atmosphere from water-soluble salts takes place upon photochemical spin-selective reactions, while the discharge of mercury compounds to sedimentary rocks takes place by means of spin-inselective reactions. Therefore, no anomaly of odd isotopes is found in sedimentary rocks, the isotope distribution in them corresponding to the classical mass-dependent effect (this conclusion should be treated with caution because the formation of the isotopic composition of the sedimentary rocks may be affected considerably by the thermodynamic isotope effect).

The nuclei of mercury isotopes differ in the mass, volume and magnetic moment, which may give rise to three isotope effects. Two effects, mass and magnetic ones, are detected reliably. The former fractionates all isotopes according to the mass of their nuclei, while the latter separates even and odd (*i.e.*, magnetic and non-magnetic) isotopes thus creating magnetic-isotope anomaly.

The volume (more precisely, the radius) of the nuclei correlates with their mass; therefore, the nuclear-volume effect cannot be separated from the mass effect. An exception is provided by ^{199}Hg and ^{201}Hg for which the difference in the radii of $\sim 10^{-7}$ Å should result in the ratio $\Delta^{199}\text{Hg}/\Delta^{201}\text{Hg} \approx 2$.

However, in all cases where this ratio was measured experimentally it was close to unity. Such ratio was to be expected of the magnetic isotope effect as the magnetic moments of these nuclei differ by only 10%. Only in a single case (Patagonia peat), the ratio $\Delta^{199}\text{Hg}/\Delta^{201}\text{Hg}$ was nearly 2. The reason for this exception is unknown; however, its physicochemical origin is unlikely.

The inversion of MIE sign observed for ^{199}Hg is incompatible with the nuclear-volume effect for which the sign inversion is impossible. These facts indicate that the contribution of the nuclear-volume effect to mercury isotopic fractionation is insignificant; the major contribution to nuclear separation is made by the other two isotope effects — the nuclear-mass and the nuclear-magnetic effects (see also Refs 21 and 22).

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