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TOXICOLOGY OF CUPRIC SALTS IN HONEYBEES. II – FEEDING BEHAVIOR FOR DIFFERENT ORGANIC SALTS AND COMPARATIVE TOXICOKINETICS OF DIETARY GLUCONATE AND SULFATE

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Worker honeybees were fed with 2M sucrose syrups pure or containing a range of added concentrations of various cupric salts. The rate of ingestion of the different syrups decreased in the following order: gluconate > asparaginate > glutamate > sulfate = glycinate > citrate. Decreasing dose-related responses were observed for aspartate, isoleucinate and sulfate, in pollensupplied or more markedly in pollen-deprivated groups, and for gluconate under pollen deprivation conditions only. Rates of ingestion for glycinate, glutamate, asparaginate and lactate were not improved by mixing an equal part of gluconate with these solutions. Glucoheptonate and pyroglutamate showed ingestion rates between asparaginate and glutamate.

The accumulation and release of copper metal in the whole body of bees was studied for sulfate and gluconate with or without pollen supply. A saturation phenomenon appeared in each case, but with higher levels for sulfate, thus limiting the concentrations retained by the bee's body, whatever the concentrations they were fed with. The kinetics of copper release from bees fed with syrups containing no added copper exhibited a sigmoidal curve, as did the percentages of copper elimination at various doses of gluconate or sulfate, reaching maxima of 97.5-98.5 %. The corresponding EC50 values decreased from 6.4 to 5.8 mM and from 7.4 to 5.6 mM respectively when 0.44 to 1.1 mM of each salt was administered, indicating low risks of excessive accumulation.

Key words: Cupric salts, worker honeybees ingestion kinetics, copper residue accumulation, whole body, Varroa jacobsoni (ectoparasite mite).

INTRODUCTION

Originating from Indonesia (Oudemans, 1904), the honeybee ectoparasite mite Varroa jacobsoni reached Russia in the year 1950 (Bregetova, 1953) and invaded western Europe twenty years later (Griffith and Bowman, 1981). For a long time, only insecticide treatments showed efficiency against the mite (Merchetti and Barbattini, 1984), which represented a potential hazard to honeybee colonies (Grobov and Mikityuk, 1981). However, according to the conjecture of Popeskovic (1984) that Varroa should carry oxygen to its cells preferably using copper-containing haemocyanin-like pigments, rather than tracheae (which are not well developed in the mite), while bees have widely developed tracheae and no oxygen-carrying pigments at all, it was shown that experimentally feeding bees with cupric salts could exhibit long term efficienty action against the parasite (Guiraud et al., 1989), at with low toxicity to honeybees (Nectoux and Bounias, 1988). Recently, an important improvement was obtained using organic cupric salts (Bounias et al., 1994), and experiments were then carried out in order to evaluate the feeding attractiveness and the toxicity of these compounds to honeybees through in vivo and in vitro studies.

The present paper reports a set of results concerning the toxicokinetic aspects of this work, including the ingestion rates, residue accumulation and elimination of organic cupric organic salts in comparison with sulfate, which was first used as a laboratory model (Popeskovic and Bounias, 1986).

MATERIAL AND METHODS

Animals. Honeybees of the local race *(Apis mellifera, mellifera L.)* were allowed to emerge from operculated combs during 12-24 hours dark incubation at 33 ± 1°C and 50 ± 5% relative humidity in a regulated chamber. They were kept one by one using soft forceps and weighed, then placed in groups of 50 or 199 in cages of 80x90x100 mm in size, provided with a graduated tube allowing the determination of ingested volumes of feeding solutions and with a hole for pollen supply. These cages were incubated in the dark at 31 ± 1°C and 60 ± 10 % relative humadity for the duration of the assays. Adult parasites were collected on living bees in groups of 26, weighed and frozen at -25°C before extraction.

Experiments. Feeding syrups were basically composed of 2 M sucrose solutions in water. The cupric salts were added in appropriate amounts corresponding to the order of magnitude of treatments used in practice, that is from 0.44 to 1.1mM for organic salts. Ingested volumes were noted daily, and any dead bees removed from the cages. At least duplicate or triplicate essays were done on cages of 100 bees each.

The rates of copper ingestion were monitored in cages of 50 to 60 bees, and the whole body concentrations of copper were determined at regular time intervals, with respect to ingested amounts. Bees, collected in samples of 5 individuals representing one replicate, were frozen at -25°C, weighed and then

ground in 5ml distilled water using a glass mortar. Varroas were ground using a 0.5 ml Potter homogenizer.

Methods. Copper was determined by atomic absorption spectrometry at 324.8 nm, using a Perkin-Elmer 5100 P spectrometer. The minimum size of samples was 10 μl. Calibration curves were established using both cupric sulfate and acetate (Sigma, research Grade) at 50, 75 and 100 μg of copper per liter.

Reagents. Cupric sulfate ($CuSO_4$, 5 H_2O) was prepared according to the so-called "Macclesfield procedure, and provided by "La Cornubia" (Bordeaux, France). The following organic salts were synthesized by "Benechim S. A.", (Lessines, Belgium), essentially free of heavy metals (less than 10μg per kg): gluconate (the mainly used compound), asparaginate, aspartate, citrate, glucoheptonate, glutamate, glycinate, isoleucinate, lactate, leucinate and pyroglutamate. Each was administered alone or in the presence of gluconate, with or without a pollen supply (ad libitum).

Statistics. Rates of ingestion were expressed in μl per bee per day (μl. $Bee^{-1} \cdot D^{-1}$), and copper metal concentrations in μg per bee ($\mu g.Bee^{-1}$.) or in μg per g of bee ($\mu g.g^{-1}$.).

Means and S. D. were calculated for the number (N) of replications given in each case.

Regression slopes (b) with their standard deviations (S.D.), and correlation coefficients (p) with their associated significances P(p) (Dagnelie, 1990) were calculated using the least squares method. Calculation of algebraic parameters of the curves was done as previously described (Bounias, 1989). Spearman's rank test was used for comparison of distribution.

Modelization. Nearly all types of time or dose-related responses can be treated in a simple way using the Hill equation as a unique model. However, the most rigorous algebraic treatment of data needs the values of the controlled parameter to be specifically chosen. When this is not possible for practical reasons, an alternative processing of the data is available whenever the values of the Hill coefficient are higher than unity (Bounias, 1994).

Let R_i be the response at value f_i of the controlled factor, R_o the value at $f_0 = 0$, and R_{lim} the asymptotic limit (either a maximum or a minimum, that is respectively: $R_0 < R_i$, and $R_0 > R_i$), F_{50} the value of the controlled parameter giving half of the maximum response, and h the Hill coefficient:

$$\pm (R_0 - R_i) = (R_{lim.} - R_0) / [1 + (F_{50}/f_i)^h] \quad (1)$$

that is: $y = Y_m / [1 + K/x)^h]$ (2)

Now, replotting equation (2) gives:

$$x = Y_m(x/y) - K/x^{h-1)} \quad (3)$$

For n>1, the second term becomes neglectable at small values of x, and the regression slope of x versus x/y gives a fairly good approximation of $R_{lim.} - R_0$. For h=1 the second term gives F_{50} (identically to the Hanes plot in enzymology) but at h>1 the system may be more difficult to solve in an accurate way.

For N pairs of data, and with r as the correlation coefficient, the standard deviation of the slope (b) is:

$$sb = [((b/r)^2 - b^2)/(N-2)]^{1/2} \quad (4)$$

RESULTS

1 - Feeding behavior.

1-1. Ingestion rates versus structure of the organic moiety. Only data expressed in ingested volumes can be used comparatively for controls and copper-containing syrups. Thus, figure 1 shows a cumulative specimen curve for pure sucrose syrup. This sigmoidal response exhibits the basic characteristics upon modelization by the Hill equation, namely: the asymptotic limit of the ingested volume: V_{max} = 41.8 ml; the time for half maximum ingestion:

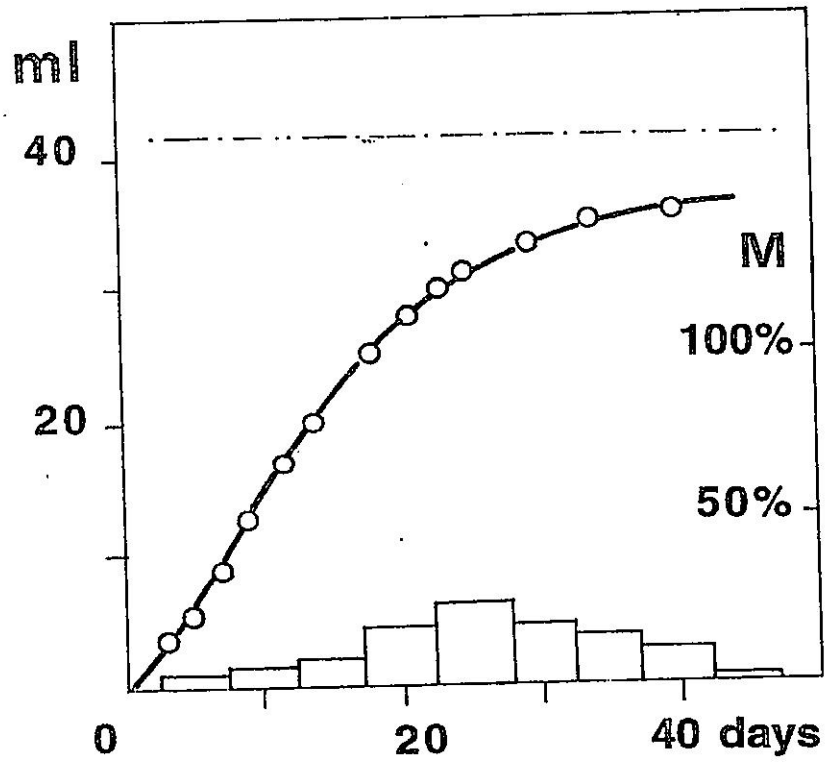

Figure 1. Time-course consumption of 2M sucrose syrup by caged honeybees. Data are given for 100 bees initially present in the cages and the distribution function of mortalities is given as a histogram centered on the classes.

T_{50} = 14.4 days; the Hill coefficient: n=1.6. This curve exhibited a nearly linear part from day 5 to day 20, but the maximum rate was given by the tangent at the inflexion point, that is:

$$R_{max} = V_{max}/(4.\ T_{50}).\ n^{-1}\ [(n-1)^{n-1}.\ (n+1)^{n+1}]^{1/n} \quad (5)$$
$$= 19.0\ \mu l.\ Bee^{-1}.\ Day^{-1}.$$

The asymptotic-like decrease of the curve may be explained by the decrease in the number of bees remaining in the cages, as shown by the distribution of mortality given in the same figure.

The obtained value of R_{max} can be very well approximated, here, by the average of the daily volume ingested per bee, that is: 18.7 ± 6.2 μl. Bee^{-1}. Day^{-1}(N = 363). Table 1 then shows a set of data comparatively obtained for a series of cupric derivatives in the presence of a pollen supply.

Table 1. Comparison of copper metal concentrations in the whole body of emerging bees, in adult workers, and in the parasite Varroa jacobsoni. Means ± S. D. are given for N independent determinations.

	(N)	$\mu g.\ Bee^{-1}$.	$\mu g.\ g^{-1}$.
emerging workers	4	0.54 ± 0.19	4.6 ± 1.9
adult workers	29	0.22 ± 0.14	2.0 ± 1.5
adult Varroa	6	0.011± 0.001	31.3 ± 5.3*

(*) Significance: P< 0.001 in comparison with bees.

Figure 2 shows specimen kinetics of copper ingestion, comparatively plotted for cupric sulfate and for 5 organic salts. Regression calculation from the linear parts of these curves gave the respective average rates of copper metal (μg) ingested per bee. Cupric gluconate exhibited the best rates, while citrate gave the lowest one. Sulfate was comparable to glycinate and lactate.

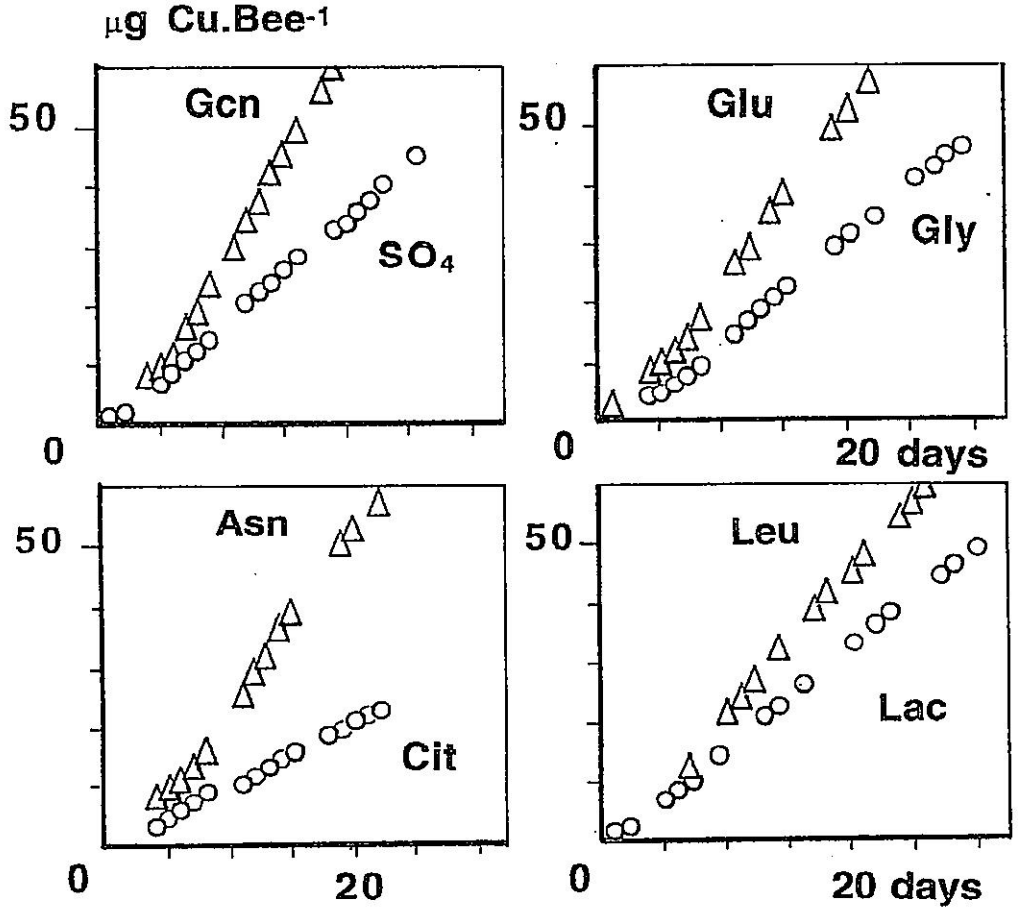

Figure 2. Comparative kinetics of copper ingestion by caged bees fed with various cupric salts. Data are expressed in cumulative μg of copper metal ingested per bee. Correlation coefficients (r) and regression equations ($y=a+[b\pm s_b].x$), calculated for the number (N) of pairs of data, were the following.
Gluconate (Gcn): ($y=-10.1 + [3.63 \pm s_b].x$). Sulfate: ($y=-1.17 + (1.75 \pm 0.012).x$). p = 0.999(N=18). Glutamate (Glu): ($y=-5.7 + (2.79 \pm 0.04).x$). p = 0.9986 (N=15). Glycinate (Gly) : ($y=-4.3 + (1.73 \pm 0.01).x$). p = 0.9998 (N=15). Asparaginate: ($y=-7.1 + (2.95 \pm 0.05).x$). p = 0.9985 (N=12). Citrate (Cit): ($y=-0.07 + (1.02 \pm 0.02).x$). p = 0.997 (N=15). Leucinate: ($y=-3.75 + (2.41 \pm 0.04).x$). p = 0.998 (N=15). Lactate (Lac): ($y=-1.1 + (1.67 \pm 0.02).x$). p = 0.999 (N=17).

Since gluconate exhibited the best results, we have examined the effects of adding gluconate in the same concentration to the other organic salts. The data are given in table 2. comparison of the observed orders in tables 1 and 2 using the coefficient of ranks of Spearman showed no correlation between the two distributions. This might indicate differences in dose-related responses.

Table 2. Ingestion rates in μl/Bee/day for 2M sucrose syrups containing 0.44 mM added organic cupric salts, and pollen supplied ad libitum. Means ± S. D. are given for the number of determinations given between parentheses. Molecular weights, water solubility, melting points, are given together with crude formulas.

Organic moiety	Formula	Molecular Weight	Water Solubility	Melting Point	Ingestion rate
None					21.5 ± 6.6 (79)
Gluconate	C_{12} H_{22} O_{14} Cu	453.8 Cu=14%	very soluble	ND	18.9 ± 7.3 (25)
Aspariganate	C_8 H_{14} O_6 N_4 Cu	325.8 Cu=19.5%	0.5%	>300°C	17.8 ± 5.4 (63)
Glutamate	C_{10} H_{16} O_8 N_2 Cu	355.8 Cu=17.85%	4%	262°C	16.6 ± 5.2 (43)
Glycinate	C_4 H_8 O_4 N_2 Cu	211.6 Cu=30%	ND	270°C	16.5 ± 4.9 (27)
Leucinate	C_{12} H_{24} O_4 N_2 Cu	323.9 Cu=19.6%	1%	270°C	16.1 ± 4.2 (35)
Citrate	C_6 H_4 O_7 Cu_2	315.2 Cu=40.3%	1%	246°C	16.0 ± 4.3 (29)
Gluco-heptonate	C_{14} H_{26} O_{16} Cu	513.9 Cu=12.35%	1%	155°C	15.9 ± 3.0 (20)
Gluconate-Gluco-heptonate	C_{13} H_{24} O_{15} Cu	483.9 Cu=13.2%	Very soluble	152°C	14.3 ± 3.6 (25)
Pyrolidone carboxylate	C_{10} H_{12} O_6 N_2 Cu	319.8		250°C	14.7 ± 5.7 (29)

QSAR-type correlation between y=ingestion rate and x=melting point:
$y = 10.9 + (0.021 \pm 0.003)x$; N=6; r=0.949; $0.001<P(r)<0.01$
(Glucoheptonate and L-Pyrolidone carboxylate excluded) or:
$y = 12.7 + (0.014 \pm 0.006)x$; N=8; r=0.675; $0.05<P(r)<0.1$
(Glucoheptonate and L-Pyrolidone carboxylate included)

1.2. Dose-response relationships. Figure 3A indicates the mean ingestion rates determined at several concentrations of gluconate, aspartate and isoleucinate. No dose effect was observed for gluconate up to 1.1 mM. A linear decrease started from 0.61 mM for aspartate, with $IC_{50} = 3.6 \pm 0.6$ mM. Isoleucinate exhibited a hyperbolic-like descending curve, with $IC_{50} = 6.9 \pm 0.15$ mM, although the high standard deviations did not allow the linearity test to

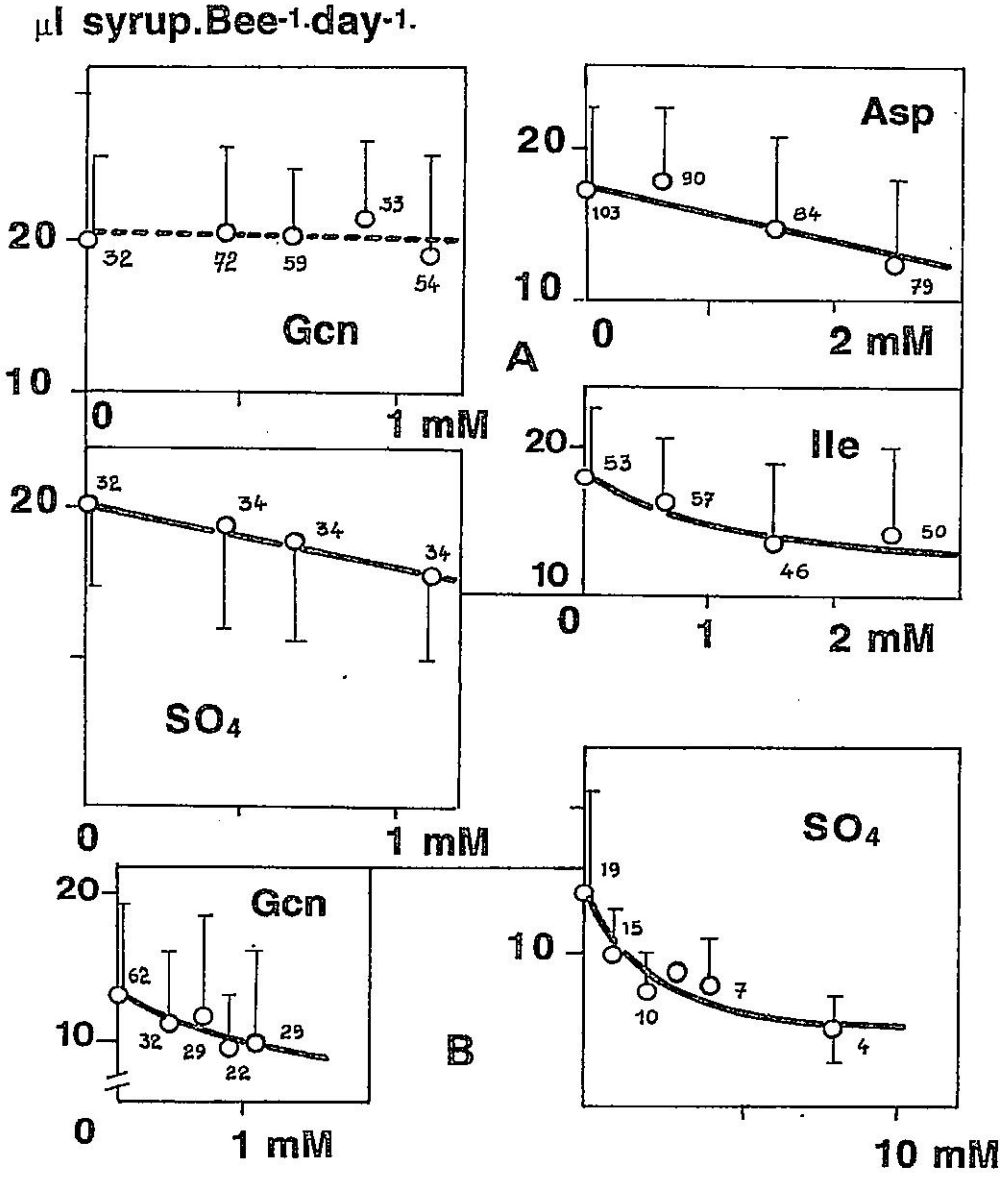

Figure 3. Dose-related ingestion rates observed for various cupric salts: A - Gluconate (Gcn), Aspartate (Asp), Isoleucinate (Ile) and Sulfate (SO_4), in the presence of pollen. B - Gluconate and Sulfate under pollen deprivation. Values are expressed in µl/bee/day. Standard deviations calculated for the number of values given for each case are represented as vertical bars.

be statistically significant. With linear regression, a lower IC_{50} = 5.1 ± 0.04mM was calculated. The curve obtained for sulfate exhibited a linear decrease, with IC_{50} = 2.6. ± 0.7 mM.

1.3. Influence of pollen deprivation. For controls fed with pure sucrose syrups, the average rate was lower than with a pollen supply: 13.3 ± 6.3 µl. Bee^{-1} Day^{-1} (N = 162). Dose-related responses were obtained for honeybees fed with cupric gluconate and sulfate in the absence of a polllen supply (Figure 3B). The former exhibited a nearly linear shape, with IC_{50} = 2.1 ± 0.8 mM,

and the latter a hyperbolic descending curve with IC_{50} = 3.8± 0.3 mM. In the hypothesis of a hyperbolic configuration for gluconate, too, the value of the constant would shift up to IC_{50} = 4.8 ± 0.7 mM.

2 - Basal Copper levels.

Individual honeybees weighed on average 109± 16mg (N=158), and the parasite 0.35 ± 0.03 mg (N = 78). Table 3 gives the corresponding levels of copper metal, including in adult foraging bees. The results expressed relatively to the weight of the animals show that the parasite body contained 7 to 15 fold more copper than emerging or adult bees, respectively.

Table 3. Ingestion rates in ml/Bee/day observed for 2M sucrose syrups with 1.1 mM added organic cupric salts, either alone or mixed with gluconate in equal amounts, and pollen supplied ad libitum. Means ± S. D. are given for (N) determinations.

Organic moieties	rate when alone	rate when mixed
Gluconate	21.8 ± 8.1 (26)	21.8 ± 8.1 (25)
Glycinate	15.5 ± 4.3 (32)	12.3 ± 4.8 (42)
Lactate	15.2 ± 4.4 (48)	10.8 ± 3.5 (9)
Glutamate	14.3 ± 6.3 (45)	14.5 ± 5.8 (46)
Asparaginate	12.4 ± 3.6 (22)	12.3 ± 4.4 (26)

3 - Toxico-kinetic studies.

The time-course concentrations of copper metal actually retained in the bee's body have been studied versus doses of gluconate fed to the animals. The data shown in figure 4-A,B,C allowed the upper limits to be calculated versus doses (figure 4-D). This last plot again exhibited the shape of a saturable phenomenon, with an asymptotic maximum:

C_{max} = 1.28 ± 0.18 μg copper / Bee (6)

The same experiment made with cupric sulfate gave the results and saturation levels shown in figure 5. In contrast with cupric gluconate, cupric sulfate exhibited a set of saturation values b decreasing with dose in a simple hyperbolic way Plotting 1/b versus doses (d) gave a statistically significant straight line ($P<0.05$):

$1/b = 0.68.\ (d) + 0.2$ (7)

that is:

$C_{max} = (0.68\ d + 0.2)^{-1}$ (8)

Copper elimination from the body of bees fed pure sucrose syrups exhibited an hyperbolic decrease as shown in figure 6. The asymptotic lower limit and the kinetic constant ET_{50} were:

C_{min} = 0.050 ± 0.014μg per Bee. (9)

ET_{50} = 4.50 ± 0.27 days. (10)

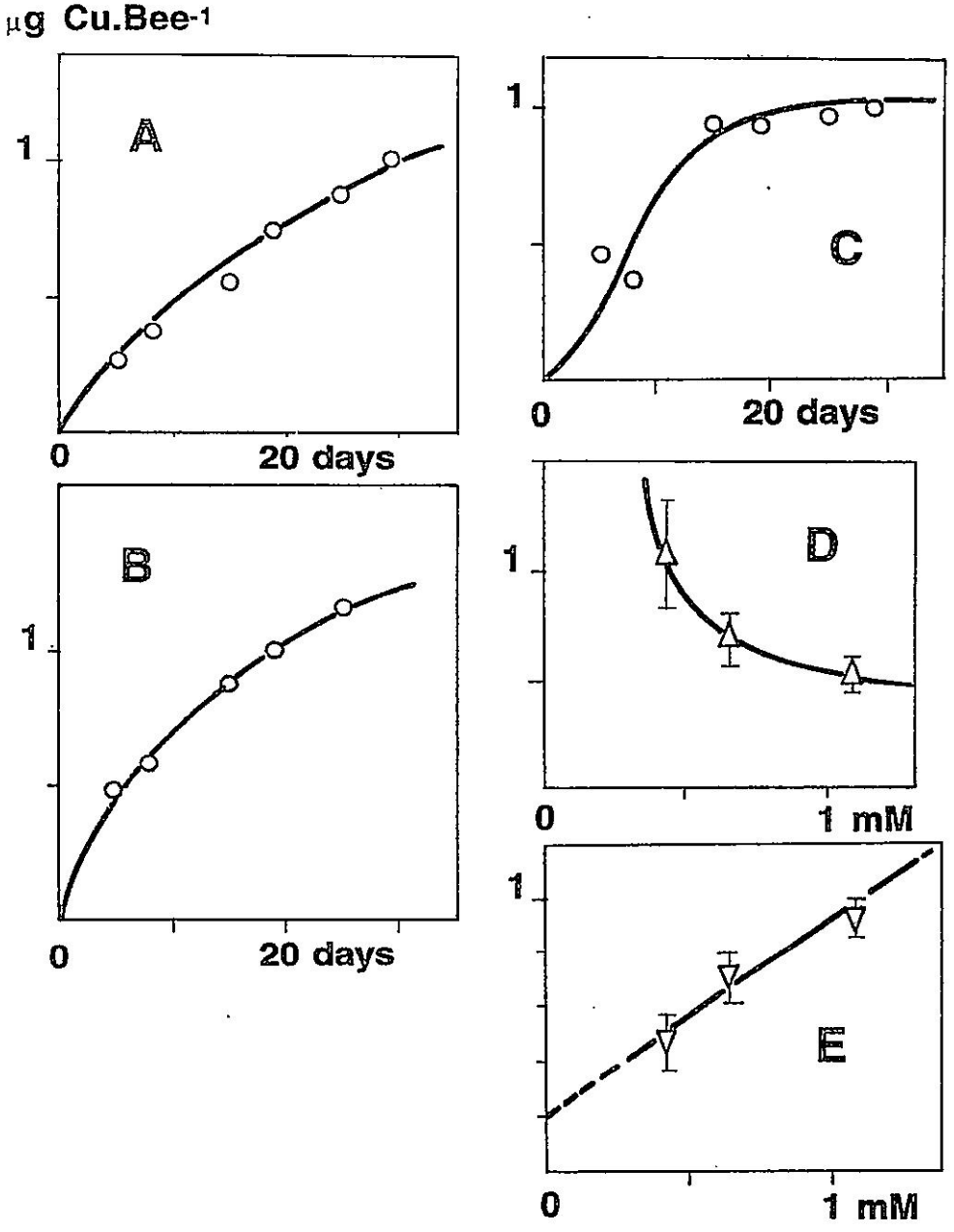

Figure 4. Time course of copper metal accumulation (μg/bee) in the whole body of bees fed with cupric gluconate:
(A) 0.44m M; (B): 0.66mM; (C) 1.1 mM.
(D) : Dose-related limits of copper accumulation.

The body concentrations (C_{at}) of copper metal retained at time (t) were then examined with respect to the ingested amounts G(t), at various concentrations (α) of cupric gluconate and sulfate. In each case, the percentage of excreted copper was estimated by parameter (T):

$$(T\alpha) = 100 \times [(C\alpha t - C_{ot})/G(t)] \quad (11)$$

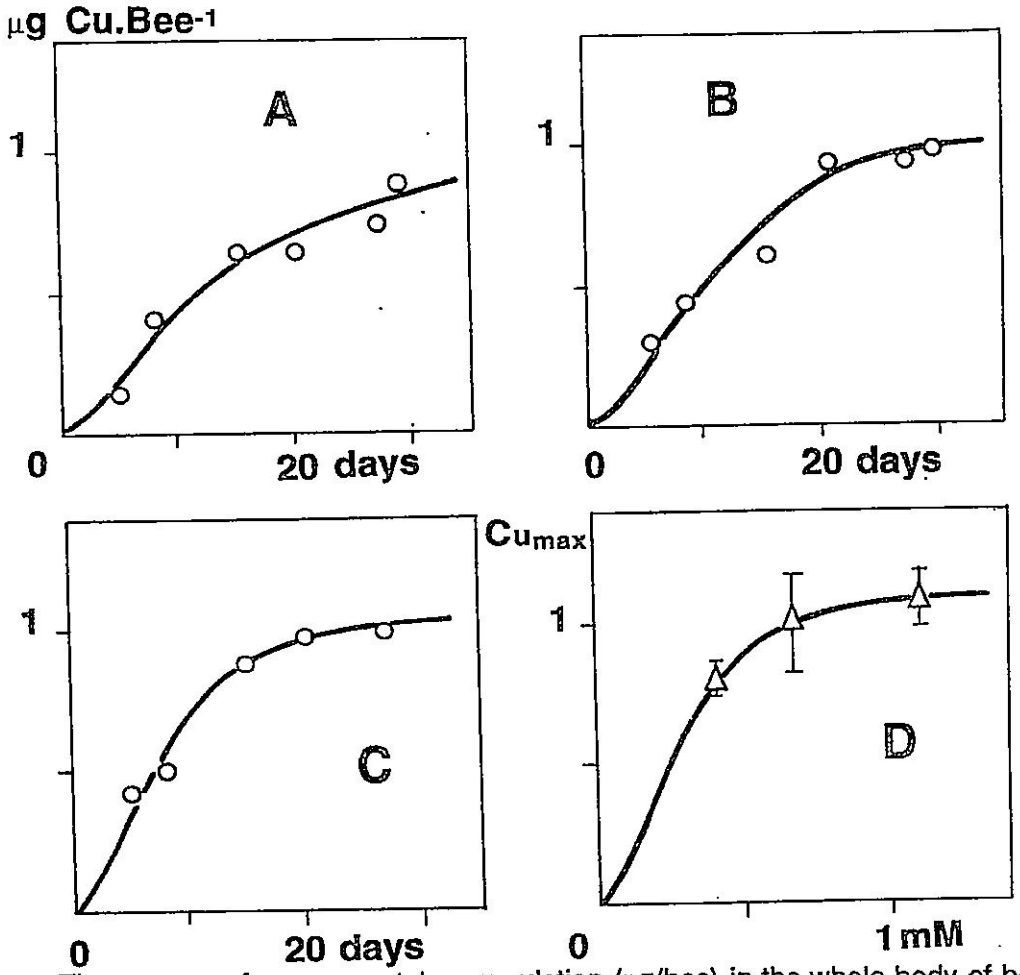

Figure 5. Time course of copper metal accumulation (µg/bee) in the whole body of bees fed with cupric sulfate: (A) 0.44m M; (B): 0.66 mM; (C) 1.1 mM.
(D) Dose-related limits of copper accumulation: direct plot (Δ).
(E) semi-reciprocal plot(Δ).

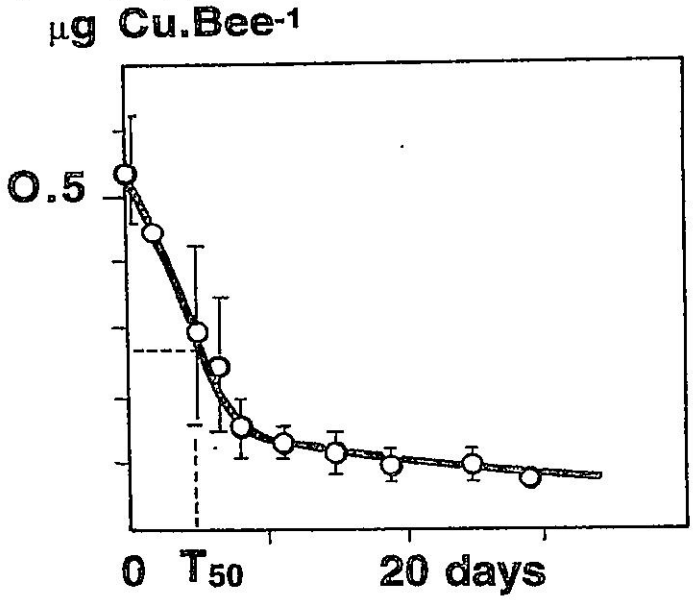

Figure 6. Clearance of copper metal from the whole body of bees fed with pure sucrose syrups, i. e. under copper deprivation. Means (µg per bee) ± S. D. (vertical bars) from triplicate experiments.

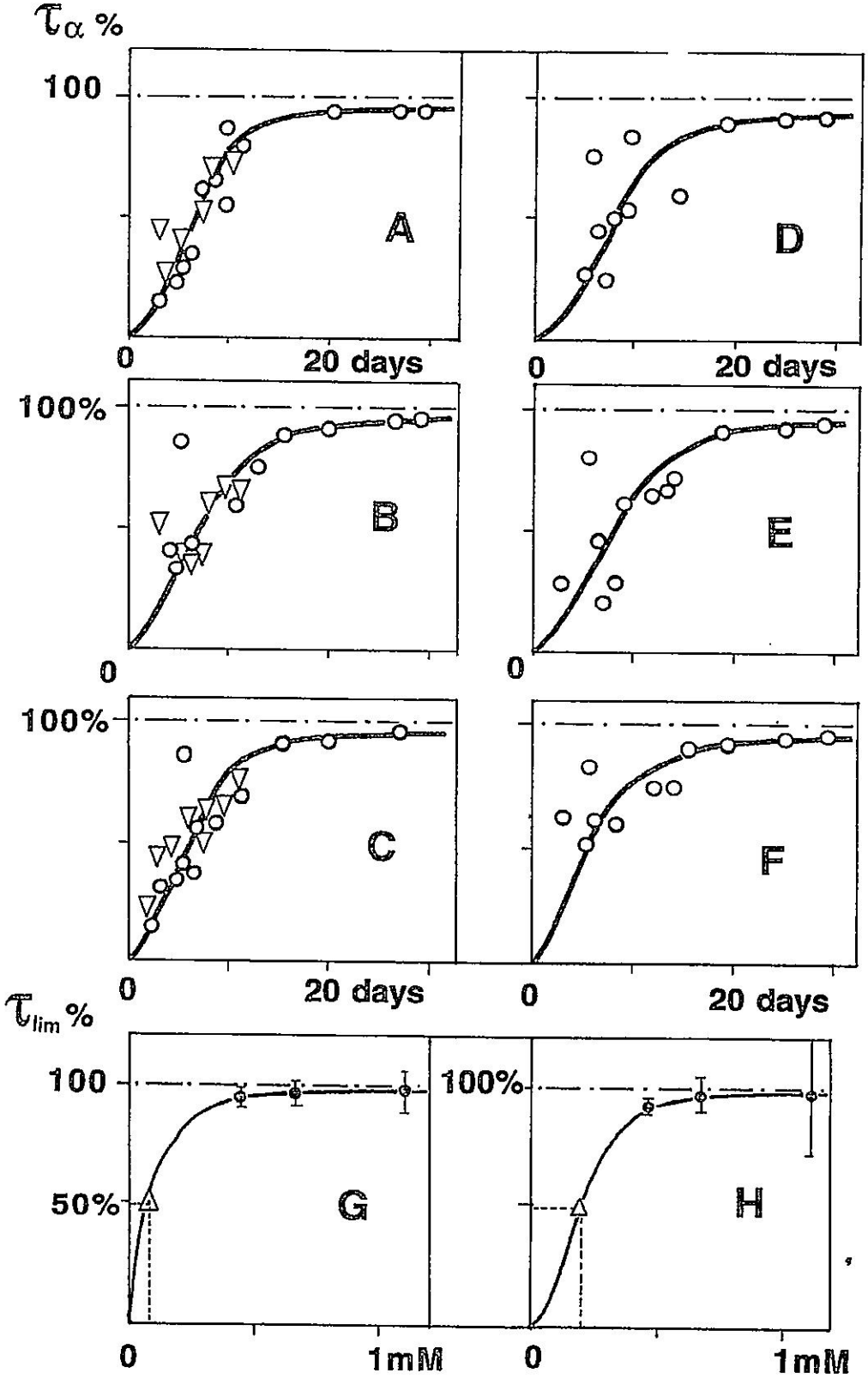

Figure 7. Time-course of copper metal excretion (Ta in ±/bee) at various doses of cupric gluconate and sulfate. (A) Gcn (0.44 mM), (B) Gcn (0.66 mM), (C) Gcn (1.1 mM). (Δ) cupric gluconate with pollen supply. (D) Sulfate (0.44 mM), (D) Sulfate (0.66 mM), (D) Sulfate (1.1 mM). Upper limits (Tlim/α in μg/bee) are plotted versus administered doses of: (G) cupric sulfate; (H) cupric gluconate;

Figure 7 shows the data obtained at various concentrations of dietary gluconate and sulfate. All curves exhibited sigmoidal shapes, whose parameters were the asymptotic limit, the Hill coefficient and the ET_{50}, that is the time at which 50 % of elimination had effectively occurred. The relevant numerical data are given in table 4. The asymptotic limits ($\tau lim/\alpha$) replotted versus doses (a) replotted versus doses (a) reached a maximum τmax = 100 % for gluconate and τmax = 98.8%for sulfate. The curve was characterized by the Hill coefficient h = 0.51 ± 0.03 and EC_{50}= 0.08 ± 0.04 mM for gluconate, while cupric sulfate gave h=0.51 ± 0.03 and EC_{50} = 0.08 ± 0.04 mM for gluconate, while cupric sulfate gave h= 3.6 ± 0.9 and EC_{50}= 0.185 ± 0.34 mM.

No influence of pollen supply was found in this case.

Table 4. Sigmoidal parameters for the rates of copper metal excretion (τa) plotted versus time, following cupric sulfate and cupric gluconate administration at various doses.

parameters:	sulfate mM			gluconate mM		
	0.44	0.66	1.1	0.44	0.66	1.1
N	9	10	9	7	9	8
ρ	0.90	0.93	0.94	0.99	0.96	0.98
a	-6.96	-4.6	-3.6	-5.4	-4.6	-5.1
b	3.47	2.47	2.11	2.9	2.6	2.9
$\pm \sigma$ b	±0.64	±0.41	±0.028	±0.19	±0.27	±0.25
$\tau_{50/\alpha}$	7.4	6.96	5.6	6.46	5.87	5.89
(mM)	±1/6	±0.40	±0.7	±0.43	±0.61	±0.57
τ_{max}(mM)		98.6 +2.3 %			100.0 + 3.2 %	+

DISCUSSION

The organic or inorganic moiety influences the feeding behaviour of the bees, with a maximum ingestion rate obtained for gluconate, which was the closest to a carbohydrate structure. For the other structures, the avoidance factors seem to be related to the acidic character of the molecules. The sulfate salt gave performances comparable to that of amino-acid salt in the presence of apollen supply, where no dose-related alteration was found for gluconate. However, pollen deprivation depressed the scores in both cases, as well as in controls. Since in field conditions bees are provided with pollen, either directly from foraging, or from storage products, this will not appear as an important problem in practice. Some of the components present in pollen may exhibit a phagostimulating effect which still operates in the presence of cupric derivatives. Gluconate, however, does not show any such effects towards other cupric derivatives.

The absorption of copper metal is one of the most striking points, since on the one hand a large enough amount should be ingested and flow in the haemolymph, in order to be accessible to the parasite, and on the other hand, concentrations in the bees' bodies should be limited to subtoxic ones. The honeybee is able to accumulate various metals, such as lead (Voget, 1989),

arsenic and cadmium (Bromenshenk et al., 1991), so that it is now currently used as a bioindicator for the monitoring of pollution levels, either of industrial or agricultural origin (Taccheo et al., 1993) or even radioactive pollution (Molzham and Assmann-Werthumüller, 1993).

Here, the upper limits exhibit saturability versus doses: the maximum levels reach the range of 1 μg copper per bee within 20 to 25 days, which may partly explain the fact that treatments but only exhibit long-term efficacy (Bounias et al., 1994). This saturable amount roughly corresponds to 0.5 g of cupric derivative given to a population of 50,000 bees in a hive, which fairly well corresponds to the effective doses, including for sulfate (Guiraud et al., 1990).

The fate of copper toxicokinetics following cupric sulfate administration also exhibited saturation properties, with high rates in all cases (fig. 7). The maximum values of these rates were close to 100%, even within the range of practically utilizable doses.

The clearance of copper (figures 6-7) exhibited characteristics rather similar to those of leas (Raes et al., 1992). The rate of copper elimination gave ET_{50} values lower, although not significantly, for gluconate than for sulfate, and the observed trend was towards a decrease with increasing doses: the respective linear regression equations were:

Gluconate: $E_{50} = 8.6 - (2.77 \pm 0.25)$ dose, $(r = -0.996)$

Sulfate: $ET_{50} = 6.6 + (0.73 \pm 0.66)$ dose, $(r = -0.74)$

The shape in the latter case may suggest an hyperbolic decrease, but without statistical significance. That both curves exhibited descending shapes means that no alteration of copper elimination is to be expected at higher doses.

Taking into account previous results showing hormetic effects for gluconate and isoleucinate (Bounias, Nectoux and Popeskovic, 1994), the feeding behaviour and toxicokinetic studies confirm why these compounds are of interest for the control of Varroa jacobsoni in honeybees with maximum safety.

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TOKSIKOLOGIJA BAKARNIH SOLI ZA PČELE
II – PONAŠANJE PREMA RAZLIČITIM ORGANSKIM SOLIMA U HRANI I KOMPARATIVNA TOKSIKOKINETIKA DIJETARNOG GLUKONATA I SULFATA

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SADRŽAJ

Pčele radilice hranjene su sa 2M šećernim sirupima, čistim ili kojima su dodavane serije koncentracija različitih bakarnih soli. Nivo digestije sirupa opada u sladećem redosledu: glukonat> asparginat> glutamat> leucinat> sulfat = glicinat> citrat. Opadanje dozama uslovljenog odgovora posmatrano je za aspartat, izoleucinat i sulfat kod polenom snabdevenih, ili još izraženije kod polenom oskudnih grupa, kao i za glukonat pod uslovima bez polena. Prednosti ingestije za glicinat, glutamat, asparginat i laktat nisu bile poboljšane mešanjem jednakih delova glukonata. Glukoheptonat i piroglutamat su pokazale vrednosti za ingestiju između asparginata i glutamata.

Akumulacija i oslobađanje bakra kao metala u celom telu pčela studiran je samo za sulfat i glukomat sa, ili bez prisustva polena. Fenomen zasićenosti se pojavljuje u svakom slučaju, ali sa višim nivoima za sulfat, limitirajući koncentracije zadržane u pčelinjem telu, ma sa kojim koncentracijama su hranjene.Kinetika nestajanja bakra iz pčela hranjenih sa sirupima siromašnijim sa bakrom ispoljila se u vidu sigmoidalne krivulje. Tako je i sa procentima eliminacije bakra pri različitim dozama glukonata ili sulfata, koje dostižu maksimum od 97,5 – 98,5%. Odgovarajuće EC50 vrednosti sukcesivno opadaju od 6,4 do 5,8 mM i od 7,4 do 5,6 mM, ako su primenjene od 0,44 do 1,1 mM, ukazujući na niske rizike za povećanje akumulacije.