History, variants and usage of the "Morrison equation" in enzyme inhibition kinetics

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Abstract

The term "Morrison equation" has varied meaning in the works published by different researchers in enzyme kinetics. This technical note summarizes the historical variants and predecessors of the "Morrison equation" as published by Ackermann & Potter (1949), Morrison (1969), Cha (1975) and Greco & Hakala (1979). Attention is also given to relatively recent textbook treatments by Copeland (2010, 2013). The disparate mathematical formalisms are first converted to a unified algebraic notation and then compared with respect the type of experimental data (i.e., either absolute or fractional rates) that are required to utilize the given algebraic form. A comparison is also made with respect to the implied inhibition mechanism, if any, as well as the number and type of optimized model parameters in nonlinear regression.

Key words: enzyme kinetics; mathematics; inhibition; tight binding; Morrison Equation

Contents

1. Introduction

A casual survey of biochemical literature on tight-binding enzyme inhibition reveals that various authors apply the term "Morrison equation" to a variety of distinct algebraic formulas.

Some variations differ only superficially, in that they can be easily converted from one to another by simple algebraic rearrangements, without altering the final results of nonlinear fit. However, other differences are substantial, in several important respects enumerated below.

1. **Input data.** Some variants call for absolute reaction rates to be used as input experimental data, whereas other variants call for fractional or relative reaction rates.
2. **Number and type of adjustable parameters.** In some cases, but not in others, the uninhibited rate (zero inhibitor concentration) is treated as an adjustable model parameter.
3. **Inhibition mechanism.** In some cases, but not in others, the particular algebraic formula implies that the inhibition mechanism is known.
4. **Algebraic form.** In some cases, but not in others, the given variant of the "Morrison equation" contains the total enzyme concentration in the denominator. This affects the results of fit.

The purpose of this technical note is to clarify and summarize the various variants of what is conventionally called the "Morrison equation" in enzyme kinetics.

In section 2 we present a detailed description of each particular variant exactly as it appears in the published literature, with appropriate bibliographic references. Here the main task was to unify the notational variations by introducing a certain standard notation, such that the variants of the "Morrison equation" can be usefully compared.

In section 3 we discuss the practical implications of choosing any particular flavor of the "Morrison equation" to analyze dose-response data that arise in the experimental study of tight-binding enzyme inhibition. We also present a recommendation on how to properly identify the algebraic model actually being used, with the aim to reduce the amount of confusion currently found in the published biochemical literature.

2. Historical survey

In this section we summarize the various algebraic forms of the Morrison equation found in the biochemical literature, as well as some of it immediate precursors (for example, the closely related Ackermann-Potter equation).

In each instance we will display the published rate equation in two forms. First, we give an exact transcription as printed in the given journal article or book. The purpose is to orient the interested reader in the originally published report. Second, we show a functional equivalent of the originally published rate equation in a standardized form. The purpose is to unify the great variety of notations used by different authors and highlight important differences as well as similarities in semantics.

In this context it is important clarify out definition of *functionally* as opposed to *algebraically* equivalent forms. In particular, functional equivalents of the Morrison equation are those that

symbol	meaning
$[E]$	equilibrium concentration of the free (unbound) enzyme
E_0	total or analytic concentration of the enzyme
$[ES]$, ...	equilibrium concentrations of various enzyme complexes
$[I]$	equilibrium concentration of the free (unbound) inhibitor
I_0	total or analytic concentration of the inhibitor
k_{cat}	rate constant for $ES \rightarrow E + P$
k'_{cat}	rate constant for $ESI \rightarrow EI + P$ (partial inhibition)
K_{i}^{*}	the apparent inhibition constant
K_{i}	dissociation constant for $EI \rightleftharpoons E + I$
K_{is}	dissociation constant for $ESI \rightleftharpoons ES + I$
K_{s}	dissociation constant for $ES \rightleftharpoons E + S$
K_{ss}	dissociation constant for $ESS \rightleftharpoons ES + S$ (substrate inhibition)
$[S]$	equilibrium concentration of free substrate; $[S] = S_0$
S_0	total or analytic concentration of the substrate
v_0	initial rate observed in the absence of the inhibitor
v_{E}	v_0/E_0 treated as an adjustable parameter in data fitting
v_{i}	initial rate observed in the presence of the inhibitor
v_{r}	relative initial rate, v_i/v_0

Table 1: Symbols utilized in standardized notation of the initial rate laws for tight binding enzyme inhibition.

lead to exactly identical behavior in actual nonlinear regression of dose-response data, given a certain particular set of optimized model parameters.

Importantly, the total or analytic concentration of the enzyme can also be considered one of those optimized parameters [1]. As a consequence certain forms of the Morrison equation are algebraically equivalent but functionally distinct, depending on whether or not the total enzyme concentration is present in the denominator of the final rate law.

Throughout this report we will use the symbols listed in *Table 1* as part of the standardized notation. The symbolism and semantics of certain kinetic constants listed in *Table 1*, for example K_{s} signifying the dissociation equilibrium constant of the Michaelis complex ES, is rooted in the *rapid equilibrium approximation* in enzyme kinetics [2].

2.1. Ackermann & Potter (1949)

Ackermann & Potter [3] derived the following initial rate equation (eqn. 13, in the original numbering, on p. 6 of ref. [3]):

$$V = -k\left[\frac{K_{\mathrm{i}}(S)}{2(K_{\mathrm{s}})} + \frac{(I_t)}{2} - \frac{(E_t)}{2}\right] \pm \sqrt{\left[\frac{K_{\mathrm{i}}(S)}{2(K_{\mathrm{s}})} + \frac{(I_t)}{2} - \frac{(E_t)}{2}\right]^2 + \frac{K_{\mathrm{i}}(S)(E_t)}{K_{\mathrm{s}}}}$$

A concordance of the original and standard symbolism is shown in the *Table 2*.

The derivations by Ackermann & Potter [3] are based on three simplifying assumptions:

1. The inhibitor is kinetically competitive with substrate.

[3]	standard notation
V	v_{i}
k	k_{cat}
(S)	S_0
(I_t)	I_0
(E_t)	E_0

Table 2: Concordance of symbols for Ackermann & Potter's [3] vs. standard notation.

2. In the absence of inhibitor the enzyme reaction follows the simple Michaelis-Menten mechanism.
3. The substrate concentration is many times larger than the Michaelis constant, $S_0 >> K_{\mathrm{s}}$, such that $1 + S_0/K_{\mathrm{s}} \approx S_0/K_{\mathrm{s}}$ and $S_0/(S_0 + K_{\mathrm{s}}) \approx 1$.

Ackermann & Potter's [3] equation can be represented in functionally equivalent standard notation as shown in Eqn (1).

$$v_i = k_{\mathrm{cat}} \frac{E_0 - I_0 - K_i \frac{S_0}{K_{\mathrm{s}}} + \sqrt{\left(E_0 - I_0 - K_i \frac{S_0}{K_{\mathrm{s}}}\right)^2 + 4E_0 K_i \frac{S_0}{K_{\mathrm{s}}}}}{2} \tag{1}$$

2.2. Morrison (1969)

Morrison [4] derived the following initial rate equation (eqn. 12, in the original numbering, on p. 272 of ref. [4]):

$$v = \frac{N}{2}\left[\sqrt{\left(\frac{1}{\sum\left(\frac{N_i}{K_i}\right)} + \frac{I_{\mathrm{t}} - E_{\mathrm{t}}}{D}\right)^2 + \frac{4\ E_t}{D\sum\left(\frac{N_i}{K_i}\right)}} - \left(\frac{1}{\sum\left(\frac{N_i}{K_i}\right)} + \frac{I_{\mathrm{t}} - E_{\mathrm{t}}}{D}\right)\right]$$

A concordance of the original and standard symbolism is shown in *Table 3*.

Because the meaning of the terms N, D, and N_i is purposely left unspecified, the Morrison's original rate equation shown above is merely a generalized "template" that cannot be used for the statistical analysis of actual experimental data.

A functionally equivalent standard notation as is shown in Eqn (2), where v_{E} depends on the particular mechanism of the un-inhibited enzyme reaction and K_{i}^* depends on the particular inhibition modality.

$$v_i = v_{\mathrm{E}} \frac{E_0 - I_0 - K_{\mathrm{i}}^* + \sqrt{\left(E_0 - I_0 - K_{\mathrm{i}}^*\right)^2 + 4E_0 K_{\mathrm{i}}^*}}{2} \tag{2}$$

Unlike Morrison's original equation, which cannot be used for data analysis without further specialization of the terms N, D and N_i, the standardized version represented by Eqn (2) can be used to fit dose-response data while treating v_{E}, K_{i}^* and (optionally [1]) E_0 as adjustable model parameters.

[4]	standard notation
v	v_i
N	numerator of rate equation in the absence of inhibitors, in Cleland's [5] formalism example: $N = k_\mathrm{cat}\ (S_0/K_\mathrm{s} + ...)$
D	denominator of rate equation in the absence of inhibitors, in Cleland's [5] formalism example: $D = 1 + S_0/K_\mathrm{s} + ...$
N_i	either "1" (for inhibitor binding to the free enzyme) or a product of certain substrate concentrations (for inhibitor binding to enzyme–substrate complexes)
K_i	either K_i (for inhibitor binding to the free enzyme) or binary dissociation constants of relevant enzyme–substrate–inhibitor complexes
I_t	I_0
E_t	E_0

Table 3: Concordance of symbols for Morrison's [4] vs. standard notation.

Morrison's equation presumably represents the "initial steady-state rate equation for *any* enzyme-catalysed reaction in the presence of a tight-binding, reversible inhibitor" [4]. However, it is important to note that the equation does not cover the possibility of *partial* tight-binding inhibition, whereby some particular enzyme–substrate–inhibitor complex retains at least a partial catalytic activity.

Because of the importance of Morrison's seminal treatise [4], let us explain the transition from the originally published to the standardized form in greater detail.

Apparent inhibition constants. The concept of the "apparent" inhibition constant seemed to have been first formally introduced by Williams & Morrison [6]. The authors display the following definition as their eqn. 9 (in the original numbering) on page 444 of their paper:

$$\text{Apparent } K_i = D/\sum (N_i/K_i)$$

With this definition of K_i^* in mind, the expression in parentheses in the originally published rate equation above can be rearranged as

$$\frac{1}{\sum\left(\frac{N_i}{K_i}\right)} + \frac{I_\mathrm{t} - E_\mathrm{t}}{D} = \frac{D/\sum (N_i/K_i) + I_t - E_t}{D} = \frac{K_\mathrm{i}^* + I_t - E_t}{D} \quad .$$

Similarly,

$$\frac{4\ E_t}{D\sum\left(\frac{N_i}{K_i}\right)} = \frac{4\ E_t\ D\sum (N_i/K_i)}{D^2} = \frac{4\ E_t\ D\ K_\mathrm{i}^*}{D^2}$$

The N/D ratio as an adjustable model parameter. After these substitutions and trivial changes in signs, the originally published rate equation can be rewritten as

$$
\begin{aligned}
v &= \frac{N}{2}\left[\sqrt{\left(\frac{K_{\mathrm{i}}^{*}+I_t-E_t}{D}\right)^2+\frac{4\,E_t\,D\,K_{\mathrm{i}}^{*}}{D^2}}-\frac{K_{\mathrm{i}}^{*}+I_t-E_t}{D}\right] \\
&= \frac{N}{D}\cdot\frac{E_t-K_{\mathrm{i}}^{*}-I_t+\sqrt{\left(E_t-K_{\mathrm{i}}^{*}-I_t\right)^2+4\,E_t\,K_{\mathrm{i}}^{*}}}{2} \quad .
\end{aligned}
$$

Importantly, the ratio N/D in Morrison's notation [4] refers to the initial steady-state reaction rate observed *in the absence of inhibitors* (see eqn. 1, in the original numbering, on p. 271 of ref. [4]). In our own standardized notation, using v_0 and E_0 (see *Table 1*) instead of v and E_t utilized in the original, the definition of N/D can be represented as

$$v_0 = E_0\frac{N}{D}$$

and equivalently

$$\frac{N}{D} = \frac{v_0}{E_0} \quad .$$

In the context of actual nonlinear fit of dose-response inhibition data, the ratio v_0/E_0 can be optionally treated as one of the adjustable model parameters. Thus, we arrive at the equivalence

$$\frac{N}{D} = v_{\mathrm{E}} \quad ,$$

which leads immediately to Eqn (2). Note that for any enzyme reaction following the familiar Michaelis-Menten catalytic mechanism [2], regardless of the inhibition modality the uninhibited initial rate is defined by the Michaelis-Menten equation,

$$v_0 = E_0\,k_{\mathrm{cat}}\,\frac{S_0}{S_0+K_s} \quad ,$$

and therefore

$$v_{\mathrm{E}} = k_{\mathrm{cat}}\,\frac{S_0}{S_0+K_s} \quad .$$

Also note that v_{E} can be treated as a distinct parameter to be determined from experimental data by nonlinear regression only in analyzing single dose-response curves, principally to determine K_{i}^{*}, E_0, or both [1]. In contrast, in modality studies, v_{E} varies with S_0 and therefore cannot be treated as a distinct kinetic parameter.

2.3. Cha (1975)

Cha [7] derived the following initial rate equation (eqn. 14, in the original numbering, on p. 2179 of ref. [7]):

$$v = \frac{k_3\,S}{2\,(K_m+S)}\left\{-\left[K_{\mathrm{i}}\left(1+\frac{S}{K_m}\right)+I_t-E_t\right]+\sqrt{\left[K_{\mathrm{i}}\left(1+\frac{S}{K_m}\right)+I_t+E_t\right]^2-4\,I_t\,E_t}\right\}$$

[7]	standard notation
v	v_{i}
k_3	k_{cat}
S	S_0
I_t	I_0
E_t	E_0
K_m	K_{s}

Table 4: Concordance of symbols for Cha's [7] vs. standard notation.

A concordance of the original and standard symbolism is shown in *Table 4*. The symbol K_{i} is used by Cha with the same meaning as listed in *Table 1*.

The derivation by Cha [7] is based on two simplifying assumptions:

1. The inhibitor is kinetically competitive with substrate.
2. In the absence of inhibitor the enzyme reaction follows the simple Michaelis-Menten mechanism.

Cha's equation is fully equivalent to Eqn (2), where v_{E} and K_{i}^* are defined by Eqn (3) and (4), respectively.

$$v_{\mathrm{E}} = k_{\mathrm{cat}} \frac{S_0}{S_0 + K_{\mathrm{s}}} \tag{3}$$

$$K_{\mathrm{i}}^* = K_{\mathrm{i}} \left(1 + \frac{S_0}{K_{\mathrm{s}}}\right) \tag{4}$$

Thus, whereas Morrison's original equation is merely a generic template, which cannot be used for data analysis without first being specialized for a particular mechanism, Cha's equation represents precisely one such *specialized* form of Morrison's template equation, applicable exclusively to competitive inhibition of a Michaelis-Menten type enzyme.

Cha's vs. Ackermann-Potter's equations. Cha [7] has explicitly addressed the relationship between his and Ackermann-Potter's rate equations. Recall that Ackermann-Potter's equation applies only when the substrate concentration happens to be *very much higher than the Michaelis constant*, $S_0 >> K_{\mathrm{s}}$. Cha has shown that under those experimetnal conditions his equation becomes identical to Ackermann-Potter's equation. The following is an exact transcript of eqn. 15, in original numbering, on p. 2179 of ref. [7]:

$$v = \frac{k_3}{2} \left\{ -\left[\frac{K_{\mathrm{i}} S}{K_m} + I_t - E_t \right] + \sqrt{\left[\frac{K_{\mathrm{i}} S}{K_m} + I_t + E_t \right]^2 - 4\, I_t\, E_t} \right\}$$

This equation is equivalent to Cha's equation above, under the conditions where $S >> K_m$. Indeed, at extremely high substrate concentrations (i.e., very much higher than K_m) the following approximation holds

$$\frac{k_3\, S}{2\,(K_m + S)} \approx \frac{k_3}{2}$$

because at $S >> K_m$, the sum $K_m + S$ becomes approximately equal to S and therefore the ratio $S/(K_m + S)$ approaches "1". Similarly,

$$K_\mathrm{i}\left(1 + \frac{S}{K_m}\right) \approx \frac{K_\mathrm{i} S}{K_m}$$

because at very high S the ratio S/K_m becomes very much larger than "1" and thus $1+S/K_m \approx S/K_m$.

2.4. Greco & Hakala (1979)

Greco & Hakala [8] derived the following initial rate equation (eqn. 8, in the original numbering, on p. 12105 of ref. [8]):

$$v_i = \frac{V_0}{2\ m_e E}\left[m_e E - I_t - K_i + \sqrt{(m_e E - I_t - K_i)^2 + 4\ K_i\ m_e E}\right]$$

This equation above is referred to Greco & Hakala [8] as an "adaptation" of an equation previously derived by Morrison [4]. A concordance of the original and standard symbolism is shown in the table below. The symbols v_i and K_i are used identically. However, see comments below on the intended vs. actual meaning of K_i as used by Greco & Hakala [8].

[8]	standard notation
V_0	v_0
K_m	K_s
k_3	k_cat
S	S_0
I_t	I_0
$m_e E$	E_0

Table 5: Concordance of symbols for Greco & Hakala's [8] vs. standard notation.

Greco & Hakala's [8] equation 8 (in original numbering) can be represented in functionally equivalent standard notation as shown in Eqn (5).

$$v_\mathrm{i} = v_0 \frac{E_0 - I_0 - K_\mathrm{i}^* + \sqrt{\left(E_0 - I_0 - K_\mathrm{i}^*\right)^2 + 4E_0 K_\mathrm{i}^*}}{2\ E_0} \tag{5}$$

Note the presence of the total enzyme concentration in the denominator of Eqn (5). This particular algebraic form is what we [1] and others have often referred to as the "Morrison equation" in published literature.

Greco & Hakala's [8] presentation contains a subtle but important simplification. In their Table 1 (original numbering on page 12105 of ref. [8]) the authors state that K_i is defined as the "dissociation constant for enzyme–inhibitor complex". This implies that

$$K_\mathrm{i} = K_\mathrm{i}^* \quad ,$$

which happens to hold only for the *pure noncompetitive* inhibition modality ($K_\mathrm{i} = K_\mathrm{is}$). Greco & Hakala's [8] purposely chose this particular inhibition modality to simplify the notation. They

encourage the reader to replace K_i with K_i^* whenever an inhibition modality other than pure noncompetitive might be involved.

Greco & Hakala [8] also presented the following initial rate equation (eqn. 9, in the original numbering, on p. 12105 of ref. [8]):

$$\frac{v_i}{V_0} = \frac{1}{2\ m_e E}\left[m_e E - I_t - K_i + \sqrt{(m_e E - I_t - K_i)^2 + 4\ K_i\ m_e E}\right]$$

Assuming that v_i/V_0 is meant to represent the *relative reaction rate* as the independent variable, a functional equivalent of Greco & Hakala's equation immediately a above can be represented as shown in Eqn (6).

$$v_\mathrm{r} = \frac{E_0 - I_0 - K_\mathrm{i}^* + \sqrt{\left(E_0 - I_0 - K_\mathrm{i}^*\right)^2 + 4E_0K_\mathrm{i}^*}}{2\ E_0} \tag{6}$$

In this case it is implied that, before actually attempting to analyze the experimental data, the observed initial rates are all divided by the initial rate observed in the absence of inhibitor. Thus the expected best-fit value of the scaled independent variable v_r is unity.

Finally, Greco & Hakala [8] presented the following initial rate equation (eqn. 10, in the original numbering, on p. 12105 of ref. [8]):

$$v_i = \frac{k_3}{2}\left[m_e E - I_t - K_i + \sqrt{(m_e E - I_t - K_i)^2 + 4\ K_i\ m_e E}\right]$$

This equation is identified as an "adaptation of one derived by Ackermann and Potter". However, note that Greco & Hakala [8] used K_i in place of Ackermann & Potter's [3] $K_\mathrm{i}S_0/K_\mathrm{s}$. As before, this implies that K_i in Greco & Hakala's [8] derivation actually represents the *apparent* inhibition constant, K_i^*, not the dissociation constant K_i as such.

2.5. Copeland (2000)

Copeland [9] presented the following rate equation (eqn. 9.6, in the original numbering, in sec. 9.3 of ref. [9]):

$$\frac{v_\mathrm{i}}{v_0} = 1 - \frac{[E] + [I] + K_\mathrm{i}^\mathrm{app} - \sqrt{\left([E] + [I] + K_\mathrm{i}^\mathrm{app}\right)^2 - 4[E][I]}}{2[E]}$$

A concordance of the original and standard symbolism is shown in the *Table 6*. The symbols v_i and v_0 are used by Copeland with the same meaning as listed in *Table 1*.

[9]	standard notation
$[I]$	I_0
$[E]$	E_0
$K_\mathrm{i}^\mathrm{app}$	K_i^*

Table 6: Concordance of symbols for Copeland's [9] vs. standard notation.

Copeland's [9] equation can be represented in functionally equivalent standard notation as shown in Eqn (6) above. Note that the dependent variable is the relative reaction rate. Thus, the data analysis begins by first dividing all observed reaction rates by the initial rate observed in the absence of the inhibitor, v_0. Consequently all relative rates v_r are scaled to span the range from zero to unity.

3. Discussion

In this section we first compare the various algebraic forms of the "Morrison Equation", with emphasis on (a) whether or not the given form contains the enzyme concentration in the denominator and (b) whether absolute or relative reaction rates are treated as the experimentally observed variable. We then present a few recommendations regarding terminology and presenting the use of the "Morrison Equation" in published works.

3.1. Comparison of variant algebraic forms

In the historical survey presented above we had identified four major published variants of the "Morrison equation":

- **Ackermann & Potter's equation Eqn (1).** This equation, as originally published, applies only to competitive inhibitors assayed under the conditions whereby the substrate concentration is very much larger than the corresponding Michaelis constant.
- **Morrison's equation Eqn (2).** As originally published, this rate equation is merely a generic template that needs to be specialized for a particular substrate catalytic mechanism (terms N and D) as well as for a particular inhibition mechanism (terms N_i and K_i).
- **Greco & Hakala's *absolute* rate equation Eqn (5).** This is the initial rate equation that we [1, 10–13] and others [14] have referred to as "the Morrison equation". Importantly, it contains E_0 in the denominator and v_0 as optimized model parameters. The experimental data are absolute reaction rates.
- **Greco & Hakala's *fractional* rate equation Eqn (6).** The experimental data are relative or fraction reaction rates, $v_r = v_i/v_0$. A functional equivalent has been been promoted by Copeland [9, 15] and subsequently adopted by many biochemists.

The most salient differences between the four algebraic variants are summarized in *Table 7*.

Regarding the practical utility of the various algebraic forms of the "Morrison equation", probably the most important differences have to do with whether or not the total enzyme concentration E_0 is present in the denominator of the given rate equation. Equally important is whether one uses either absolute or fractional rates.

3.1.1. The effect of E_0 in the denominator

In the context of nonlinear regression, Morrison's Eqn (2) contains at least two and sometimes three optimized model parameters. The two parameters that always must be optimized in Eqn (2) are v_E and the apparent inhibition constant K_i^*. Under certain conditions one must also determine from the data the total enzyme concentration E_0, as the third adjustable model parameter [1][14]. Note that the physical meaning of v_E is defined as the ratio of v_0/E_0, but also note that the uninhibited initial rate v_0 as such is *not* considered to be one of the optimized model parameters:

$$v = \frac{v_{\mathrm{E}}}{2} \ldots \text{ etc.} = \frac{(v_0/E_0)}{2} \ldots \text{ etc.} \qquad \text{Morrison}$$

In contrast, Greco & Hakala's absolute rate Eqn (5) – which we [1] and others [14] frequently refer to as "the Morrison equation" – differs from Morrison's original Eqn (2) in that Eqn (5) does contain E_0 in the denominator and it does involve the control reaction rate v_0 as one of the optimized model parameters:

$$v = \frac{v_0}{2\,E_0} \ldots \text{ etc.} \qquad \text{Greco \& Hakala}$$

It would seem natural to expect that the the "Morrison" and "Greco & Hakala" fragments shown immediately above should produce the same results in data fitting, because purely algebraically

$$\frac{(v_0/E_0)}{2} = \frac{v_0}{2\,E_0} \quad .$$

Contrary to the naive expectation, Greco & Hakala's [8] found that Eqns. (2) and (5) produced significantly different results in the analysis of experimental data.

Specifically, Greco & Hakala's absolute rate Eqn (5) [8] produced a significant number of grossly erroneous results, which were characterized by "negative" inhibition constants (see Table III on p. 12107 of ref. [8], third row, last column, value "7" in parentheses). Of course in real world inhibition constants can never be negative, so the "negative" results obtained by using Eqn (5) were clearly an artifact.

In contrast, under exactly identical conditions, Ackermann & Potter's equation Eqn (1) produced no physically meaningless "negative" inhibition constants (see Table III on p. 12107 of ref. [8], first row, last column, value "0" in parentheses). In other words, all simulated data sets in the Monte-Carlo study based on Ackermann & Potter's equation Eqn (1) produced physically meaningful results.

Importantly, Ackermann & Potter's equation Eqn (1) and Morrison's equation Eqn (2) can be viewed as functionally equivalent in that Eqn (1) is a specialization of Eqn (2), in particular one that applies specifically in the case of Michaelis-Menten type enzyme inhibited by a competitive inhibitor.

Ref.	Eqns.	Mechanism	S_0	Rates	$1/E_0$
[3]	(1)	competitive	$>> K_{\mathrm{m}}$	absolute	no
[4]	(2)	any	any	absolute	no
[7]	(2), (3), (4)	competitive	any	absolute	no
[8], eqn. $8^{(a)}$	(5)	noncompetitive	any	absolute	yes
[8], eqn. $9^{(a)}$	(6)	noncompetitive	any	fractional	yes
[9]	(6)	any	any	fractional	yes

$^{(a)}$ Original numbering.

Table 7: Comparison of variant algebraic forms of the "Morrison equation". The right-most column specifies where or not the enzyme concentration is found in the denominator. For further details see text.

In this respect Morrison's original Eqn (2) was found in Greco & Hakala's Monte-Carlo studies [8] *significantly more useful* than what is frequently called "the Morrison equation" in published literature, i.e., Eqn (5) derived by Greco & Hakala's [8].

The implication of these findings appears to be that we should probably abandon "the Morrison equation", as derived by Greco & Hakala (1979), in favor of Morrison's (1969) original equation, either specialized for the given catalytic and inhibition mechanism or simply generalized by the use of the apparent inhibition constant K_i^*.

3.1.2. Utilizing absolute vs. fractional rates

Many authors (see ref. [16] as one of many examples) have been using Greco & Hakala's relative of *fractional* rate Eqn (5) [8], as advocated by Copeland [9, 15].

In this context it is very important to realize that the very use of Eqn (5), for example in the form presented by Copeland,

$$\frac{v_i}{v_0} = 1 - \frac{\left([E] + [I] + K_i^{app}\right) - \sqrt{\left([E] + [I] + K_i^{app}\right)^2 - 4[E][I]}}{2\,[E]}$$

silently implies that the control data point (v_0) is entirely *free of random experimental error*. As a matter of course, this simplifying assumption is never satisfied exactly because *all* data points, including v_0, are subject to random experimental noise.

If the data quality is exceptionally high (e.g., less than 3% random error in measuring initial rates) nsisting on the unrealistic assumption that v_0 is error-free will not cause undue damage. However, simple back-of-the-envelope computations show that a realistically large 10% error in v_0 can cause up to 50% systematic distortion in the best-fit value of K_i^*.

These preliminary results strongly suggest that the use of absolute reaction rates is very much preferable in comparison with relative or fractional rates.

3.2. Conclusions and recommendations

Published research on tight-binding enzyme inhibition apparently contain a profusion of disparate and in some cases confusing terminology regarding the terms "Morrison equation" and, in some cases "Ackermann and Potter equation".

Some authors use one or both of these terms, but do not actually show the either equation they used or publish a bibliographic reference. Other authors provide a bibliographic reference but do not present any actual mathematical model that thay used to analyze their concentration vs. velocity data.

For example, Borges et al. [17] state that the "Ackermann and Potter equation for tight-binding inhibitors (Cha, 1975) was fitted to the initial velocities." [17] The authors do not actually present their mathematical model. On close examination it appears that the authors used the "Morrison equation" derived by Greco & Hakala Eqn (5). However, they refer to it as the "Ackermann and Potter equation", with a reference to the paper by Cha [7].

3.2.1. Reporting results of least-squares fit

To alleviate such pervasive confusion, a following set of recommendations can be made for researchers involved with the study of tight-binding enzyme inhibition.

1. Write down the actual rate equation that was used to analyze the experimental data, rather than relying on generic terms such as "Morrison equation".

2. State whether the experimental data were raw reaction rates (v_0 and v_i) or fractional rates ($v_r = v_i/v_0$).
3. State whether the enzyme concentration E_0 was treated as fixed constants or as an optimized model parameter [1].

3.2.2. What, if anything, is the "Morrison Equation"?

Based on the historical survey presented in section 2, it is clear that the rate equation originally derived by Morrison (eqn. 12, in the original numbering, on p. 272 of ref. [4]) is not suitable for the statistical analysis of any actual experimental data. This is because the generic terms N, D, and N_i utilized by Morrison, presumably on direct recommendation of W. W. Cleland[1], must first be specialized for a particular catalytic and inhibition mechanism and only then Morrison's original equation can be used in practice.

From this fact it would seem to follow that, strictly speaking, *no* particular rate equation currently in use to analyze concentration–response data in tight-binding inhibition should be called "the Morrison equation", because (to repeat for emphasis) Morrison's equation is merely a generic or abstract template, not a concrete rate equation.

Over time, various researchers came up with various specializations of Morrison's generic template equation. For example, Cha [7] derived a specialization applicable to any enzyme that follow the simple Michaelis-Menten mechanism and is inhibited in a kinetically competitive fashion. Therefore it would seem proper to call this specialization "the Cha equation" or "Cha's equation".

Importantly, in the context of nonlinear regression analysis, Morrison's original rate equation, as well as its immediate predecessor the "Ackermann-Potter equation" [3] and the "Cha equation" [7] as the immediate successor, all contain $v_E = N/D$ as one of the adjustable model parameters. Note that the physical meaning of v_E is equivalent to the un-inhibited reaction rate *divided by the total enzyme concentration*. Therefore E_0 does *not* appear in the denominator.

Greco & Hakala [8] came up with a particular algebraic reformulation of Morrison's original equation, which consisted of introducing the *uninhibited reaction rate*, v_0, as one of the adjustable model parameters. This has lead to E_0 appearing, for the first time, in the denominator of the resulting formula. Furthermore, Greco & Hakala [8] experimented with using either absolute reaction rates or fractional rates as input data.

Greco & Hakala' *absolute* rate equation (eqn. 8, in the original numbering, on p. 12105 of ref. [8]) is what we [1] and others [14] have been referring to as "the Morrison equation". On reflection, that particular rate equation should be more appropriately called the **Greco-Hakala absolute rate equation**.

Greco & Hakala' *relative* rate equation (eqn. 9, in the original numbering, on p. 12105 of ref. [8]) is what Copeland [9] [15] and others [16] have been referring to as "the Morrison equation". On reflection, that particular rate equation should be more appropriately called the **Greco-Hakala relative rate equation**.

The main reason why both the absolute and the relative variant of the **Greco-Hakala equation** deserves to be distinguished from Morrison's generic template is the presence of E_0 in the denominator. This is not merely a formal distinction, but rather it leads to a fundamentally different behavior in nonlinear regression, as documented in the original paper [8] and described in section 2.4.

[1] See the Acknowledgments section in [4]: "Thanks are due to Dr. W. W. Cleland for his comments on the manuscript which included the suggestion that certain equations be given in general form."

In summary, there is no "Morrison equation" that can be applied to the analysis of actual experimental data. The two particular algebraic formulas derived by Greco & Hakala [8], which are conventionally referred to as the "Morrison equation", are functionally distinct from Morrison's original equation [4] in that they produce different results in statistical analysis. Therefore the two initial rate equations currently in frequent use, namely Eqns (5) and (6) as well as their respective functional equivalents, should be more rightfully called the "Greco-Hakala equations".

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