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Ibio-Number Assay and Erythropoietin Bioactivity: Comparison of the Calculated and the Stated Potencies of the Biological Reference Preparations of Erythropoietin

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Abstract

The potencies of the biological reference preparations of erythropoietin, assigned by the European Pharmacopoeia Commission on the basis of the normocythaemic and polycythaemic mouse bioassay for erythropoietin, have retrospectively been calculated by the author via the Ibio-number assay, a physicochemical assay based on capillary zone electrophoresis data that allows to calculate the potency of erythropoietin medicinal products.

The retrospective analysis by the author of the capillary electrophoresis data of three collaborative studies published in 2004, 2007 and 2015 has revealed that the potencies assigned for erythropoietin reference preparations batch 1 and batch 2 (~130.0 IU/µg, each) have been stated ~5 % too low respectively ~10 % too low, whereas the potency stated for erythropoietin reference preparation batch 3 (~141.1 IU/µg) was confirmed (difference to the stated potency = -1.2%) and therefore free of doubts.

Thus, erythropoietin medicinal products that have been calibrated against erythropoietin reference preparation batch 1 or batch 2 have been subject to the same error which was, however, within the error of the mouse bioassay and therefore not crucial.

With respect to erythropoietin concentrated solution batch release according to the European Pharmacopoeia, the very broad criteria for erythropoietin identification via capillary zone electrophoresis (which is based on broad ranges defined for the various erythropoietin isoforms) could be replaced by a single and quite narrow Ibio-number range, which would provide a significant increase in assay precision and accuracy and hence in drug safety.

Moreover, the Ibio-number assay could be a candidate physicochemical assay to replace the mouse bioassay in the quality control of erythropoietin batch release.

Keywords Bioactivity; Bioassay; Biological reference preparation; Capillary zone electrophoresis; Erythropoietin; Potency

Abbreviations

BRP: Biological Reference Preparation; cBRP: Candidate BRP; CRS: Chemical Reference Substance; cCRS: candidate CRS; CV: Coefficient of Variation; CZE: Capillary Zone Electrophoresis; EDQM: European Directorate for the Quality of Medicines; EPO: Erythropoietin; Ibio-number: Isoform number calculated via the peak numbering in CZE; in: Individual isoform number shares; IS: International Standard; IU: international unit; MV: Mean value; NIBSC: National Institute for Biological Standards and Control; Ph. Eur.: European Pharmacopoeia; pn: Peak area percent shares; ref(s): Reference(s)

Introduction

Recombinant human erythropoietin is a biotechnologically produced hormone which stimulates human red blood cell growth and is therefore marketed worldwide for the treatment of anemia. The biological activity of the erythropoietin medicinal products is determined via in vivo assays in mice which are known to be highly inaccurate (CV ≈ 25% [1,2], ≈ 20% [3,4]; uncertainty 15-30% [5] as stated by Zimmermann et al. [3]). Therefore, there are ongoing efforts to replace the highly contested (consumption of animals) and highly variable polycythaemic and normocythaemic mouse bioassays in the quality control of erythropoietin by more precise and more accurate physicochemical methods.

One of these proposed alternatives is the Ibio-number assay [6], a physicochemical assay based on the CZE data gathered according to Ph. Eur. [7], which has been shown to predict the bioactivity of EPO medicinal products with high precision and accuracy [6].

In principle, the assay has already been introduced in 2006 [8,9], however has only recently been termed as such [6], when the assay was applied to calculate the potencies of various EPO drug substance and drug product samples, including the candidate EPO biological reference preparation batch 3.

Whereas the "Ibio-number assay" [6] uses the bioactivity of EPO isoforms for potency calculation, the "I-number assay" [10] (another alternative physicochemical assay) uses the peak numbering of EPO isoforms for bioactivity calculation and is calibrated against the stated

bioactivity of EPO BRP3, the potency of which – in contrast to EPO BRP1 and EPO BRP2 – was without any doubt [11].

The current "Ibio-number" study in a way parallels the "I-number" study reported elsewhere [11] as it is based on the same set of CZE data gathered in various collaborative studies designed to establish EPO BRP1 [12], EPO BRP2 [13], EPO BRP3 [14] and the EPO chemical reference substance [15]. However, the current study extends its scope to EPO BRP batch 4 [16] and to the WHO 3rd International Standard for Erythropoietin, recombinant, for bioassay (11/170) [17].

Materials and Methods

Materials

EPO BRP1, cBRP2, BRP2, cBRP3, BRP3 and cCRS were as described in collaborative studies published in 1997 [12], 2004 [13], 2007 [14] and 2015 [15], and the CZE data were taken from the latter three studies. In each of these studies, the (c)BRPs consisted in 50:50 (weight / weight) blends of epoetin-alpha and epoetin-beta. The stated potencies of BRP1 and BRP2 were 32,500 IU/vial, each, where each vial contained approximately 250 µg EPO [12,13], equivalent to 130,000 IU/mg or 130.0 IU/µg. The stated potency of BRP3 was 35,280 IU/vial, where each vial contained approximately 250 µg EPO [14], equivalent to 141,120 IU/mg or 141.1 IU/µg.

EPO BRP batch 4 again was an epoetin α/ß 1:1 mixture – with a stated potency of 13 000 IU/vial [16]. "This batch was prepared with the same (for EPO-β) or similar (for EPO-α) starting materials, carrier buffer and production process as were used to produce the BRP3 but with a lower content, i.e. around 100 µg of EPO per vial instead of 250 µg/vial for BRP3" [16].

The 3rd WHO International Standard for Erythropoietin, recombinant, for bioassay (NIBSC code: 11/170) contains 1650 IU per ampoule, where each ampoule contains approximately 11 µg of recombinant human EPO from the USA [17].

Methods

The Ibio-numbers of these EPO materials were calculated as earlier described [6]: "Ibio is defined as the sum of the products of the individual CZE peak area percent shares (pn) of the EPO isoforms (n = 1-8) and isoform factors Fi corresponding with the respective isoform bioactivities [8,9] (Formula 1 [6]):

$$Ibio = p1 \times F1 + p2 \times F2 + p3 \times F3 + p4 \times F4 + p5 \times F5 + p6 \times F6 + p7 \times F7 + p8 \times F8$$ Formula 1

For epoetin alfa and epoetin beta, the factors Fi were derived from the bioactivities of the individual isoforms as published by Amgen for epoetin alfa [18] (providing Ibio_A) (Tables 1a-5a) respectively Roche for epoetin beta [19] (providing Ibio_R) (Tables 1b-5b). The final Ibio-number was the arithmetic mean of Ibio_A and Ibio_R (cp. Tables 1c-5c). As the isoform bioactivities were published as "units/mg erythropoietin polypetide" [18] respectively "IU/mg protein" [19], the 'primary' Ibio-numbers were "multiplied by the factor 0.60 polypetide/mg erythropoietin glycoprotein to give specific activities expressed as units/mg erythropoietin glycoprotein" [18] respectively the 'final' Ibio-numbers. Isoforms 1-8 (where applicable) were used for Ibio-number calculation [6].

Ibio-number calculation for cBRP3 was described in details elsewhere [6]. The Ibio-numbers of BRP1, cBRP2, BRP2, BRP3 and cCRS were calculated alike from the CZE data provided in the corresponding collaborative studies [13-15], which is shown in Tables 1-5.

The Ibio-numbers retrospectively calculated by the author from the CZE data of these studies were used to assess the inter-laboratory precision of the assay. The accuracy of the assay was determined by relating the calculated Ibio-numbers to the stated bioactivities of the BRPs set 100%, each.

Peak No.	Amgen IU/µg polypeptide		Lab 2		Lab 3		Lab 8		Lab 9		Lab 10		Lab 11	
		Factor	pn	in	pn	in	pn	in	pn	in	pn	in	pn	in
1	50.3	0.50	0.4	0.2	0.8	0.4	1.0	0.5	0.8	0.4	0.8	0.4	1.0	0.5
2	70.6	0.71	1.9	1.3	2.5	1.8	3.1	2.2	2.2	1.6	2.4	1.7	2.7	1.9
3	96.6	0.97	5.9	5.7	6.0	5.8	6.9	6.7	5.6	5.4	6.2	6.0	6.2	6.0
4	170.3	1.70	18.4	31.3	18.3	31.2	17.8	30.3	19.1	32.5	17.7	30.1	19.0	32.4
5	255.8	2.56	29.6	75.7	29.0	74.2	28.2	72.1	30.1	77.0	28.8	73.7	28.8	73.7
6	258.4	2.58	28.7	74.2	27.9	72.1	27.5	71.1	24.4	63.0	28.5	73.6	27.8	71.8
7	258.7	2.59	14.4	37.3	14.2	36.7	14.1	36.5	16.1	41.7	14.6	37.8	13.7	35.4
8	205.8	2.06	0.7	1.4	1.3	2.7	1.4	2.9	1.8	3.7	0.9	1.9	0.8	1.6
Ibio_A_primary =			227.1		224.8		222.2		225.3		225.2		223.3	
Ibio_A_final =			136.3		134.9		133.3		135.2		135.1		134.0	
Mean Ibio_A = 134.8 ± 1.0			CV = 0.8%		(n = 6)									

Table 1a: BRP1 Ibio-number calculation and inter-laboratory precision. CZE data derived from Table 3a of Behr-Gross et al. 2004 [13] – Isoform distribution (in %) of BRP1, using isoform bioactivities published by Amgen [18].

Peak No.	Roche IU/µg protein		Lab 2		Lab 3		Lab 8		Lab 9		Lab 10		Lab 11	
	#)	Factor	pn	in	pn	in	pn	in	pn	in	pn	in	pn	in
1	19	0.19	0.4	0.1	0.8	0.2	1.0	0.2	0.8	0.2	0.8	0.2	1.0	0.2
2	40	0.40	1.9	0.8	2.5	1.0	3.1	1.2	2.2	0.9	2.4	1.0	2.7	1.1
3	75	0.75	5.9	4.4	6.0	4.5	6.9	5.2	5.6	4.2	6.2	4.7	6.2	4.7
4	150	1.50	18.4	27.6	18.3	27.5	17.8	26.7	19.1	28.7	17.7	26.6	19.0	28.5
5	200	2.00	29.6	59.2	29.0	58.0	28.2	56.4	30.1	60.2	28.8	57.6	28.8	57.6
6	280	2.80	28.7	80.4	27.9	78.1	27.5	77.0	24.4	68.3	28.5	79.8	27.8	77.8
7	400	4.00	14.4	57.6	14.2	56.8	14.1	56.4	16.1	64.4	14.6	58.4	13.7	54.8
8	205.8	2.06	0.7	1.4	1.3	2.7	1.4	2.9	1.8	3.7	0.9	1.9	0.8	1.6
Ibio_R_primary =			231.5		228.7		226.0		230.5		230.0		226.3	
Ibio_R_final =			138.9		137.2		135.6		138.3		138.0		135.8	
Mean Ibio_R = 137.3 ± 1.4			CV = 1.0%		(n = 6)									

Table 1b: Using isoform bioactivities published by Roche [19]. #) Specific activity of isoform peak no. 8 as published by Amgen [18] as the specific activity of this isoform of Roche [19] was not available.

Lab no.		2	3	8	9	10	11	cp. Table 1a
BRP1	A	136.3	134.9	133.3	135.2	135.1	134.0	
	R	138.9	137.2	135.6	138.3	138.0	135.8	cp. Table 1b
	Mean	137.6	136.1	134.5	136.7	136.5	134.9	
Mean of means	136.0 ± 1.2	CV = 0.9%	(n = 6 labs)					

Table 1c: Mean of means. A: Ibio-numbers calculated using isoform factors deduced from ref [18], R: Ibio-numbers calculated using isoform factors deduced from ref [19].

Outliers according to Grubbs [20] respectively Dixon [21], as retrospectively identified by the author in the collaborative studies upon Ibio-number calculation, are specified in footnotes to the Tables and disregarded in the current study.

As no CZE data were communicated for EPO BRP batch 4, the Ibio-number of EPO BRP batch 4 was regarded identical to the Ibio-number of EPO cCRS, as these two materials were prepared from the same batch produced at the NIBSC, UK, in 2012 [16].

As, likewise, no CZE data were available for the 3rd IS, which consists of epetin alfa, the epoetin alfa secondary standard from Centocor was used, the CZE data of which have been gathered in a kind of limited validation (determination of repeatability and intermediate precision) [22]; the corresponding Ibio-number calculation has been published elsewhere [6].

Peak No.	Amgen IU/µg polypeptide		Lab 2		Lab 3		Lab 8		Lab 9		Lab 10		Lab 11		Lab 12	
		Factor	pn	in	pn	in	pn	in	pn	in	pn	in	pn	in	pn	in
1	50.3	0.50					0.3	0.2	0.5	0.3	0.4	0.2	0.3	0.2	0.2	0.1
2	70.6	0.71	1.0	0.7	1.0	0.7	1.6	1.1	0.9	0.6	1.3	0.9	1.5	1.1	1.0	0.7
3	96.6	0.97	4.5	4.3	4.9	4.7	5.1	4.9	3.8	3.7	4.6	4.4	5.2	5.0	5.3	5.1
4	170.3	1.70	16.8	28.6	16.8	28.6	16.2	27.6	17.3	29.5	15.4	26.2	19.0	32.4	17.3	29.5

5	255.8	2.56	27.1	69.3	27.2	69.6	26.3	67.3	27.8	71.1	26.8	68.6	28.7	73.4	26.9	68.8
6	258.4	2.58	30.4	78.6	29.7	76.7	30.0	77.5	29.4	76.0	30.4	78.6	30.0	77.5	29.6	76.5
7	258.7	2.59	18.6	48.1	18.3	47.3	18.6	48.1	18.6	48.1	19.2	49.7	15.2	39.3	18.1	46.8
8	205.8	2.06	1.7	3.5	2.1	4.3	1.8	3.7	1.8	3.7	1.9	3.9	0.1	0.2	1.7	3.5
Ibio_A_primary =			233.2		232.0		230.4		232.9		232.5		229.1		231.0	
Ibio_A_final =			139.9		139.2		138.2		139.8		139.5		137.4		138.6	
Mean Ibio_A = 138.9 ± 0.9			CV = 0.6%				(n = 7)									
Mean Ibio_A = 139.2 ± 0.7			CV = 0.5%				(n = 6)									

Table 2a: cBRP2 Ibio-number calculation and inter-laboratory precision. CZE data derived from Table 3b of Behr-Gross et al. 2004 [13] – Isoform distribution (in %) of cBRP2, using isoform bioactivities published by Amgen [18].

Peak No.	**Roche IU/µg protein**		**Lab 2**		**Lab 3**		**Lab 8**		**Lab 9**		**Lab 10**		**Lab 11**		**Lab 12**	
	#)	Factor	pn	in	pn	in	pn	in	pn	in	pn	in	pn	in	pn	in
1	19	0.19					0.3	0.1	0.5	0.1	0.4	0.1	0.3	0.1	0.2	0.0
2	40	0.40	1.0	0.4	1.0	0.4	1.6	0.6	0.9	0.4	1.3	0.5	1.5	0.6	1.0	0.4
3	75	0.75	4.5	3.4	4.9	3.7	5.1	3.8	3.8	2.9	4.6	3.5	5.2	3.9	5.3	4.0
4	150	1.50	16.8	25.2	16.8	25.2	16.2	24.3	17.3	26.0	15.4	23.1	19.0	28.5	17.3	26.0
5	200	2.00	27.1	54.2	27.2	54.4	26.3	52.6	27.8	55.6	26.8	53.6	28.7	57.4	26.9	53.8
6	280	2.80	30.4	85.1	29.7	83.2	30.0	84.0	29.4	82.3	30.4	85.1	30.0	84.0	29.6	82.9
7	400	4.00	18.6	74.4	18.3	73.2	18.6	74.4	18.6	74.4	19.2	76.8	15.2	60.8	18.1	72.4
8	205.8	2.06	1.7	3.5	2.1	4.3	1.8	3.7	1.8	3.7	1.9	3.9	0.1	0.2	1.7	3.5
Ibio_R_primary =			246.2		244.4		243.5		245.3		246.6		235.5		242.9	
Ibio_R_final =			147.7		146.6		146.1		147.2		147.9		141.3		145.8	
Mean Ibio_R = 146.1 ± 2.3			CV = 1.6%				(n = 7)									
Mean Ibio_R = 146.9 ± 0.9			CV = 0.6%				(n = 6)									

Table 2b: Using isoform bioactivities published by Roche [19]. #) Specific activity of isoform peak no. 8 as published by Amgen [18] as the specific activity of this isoform of Roche [19] was not available. Note: Lab 11 proved to be an outlier according to Grubbs (α = 0.05) and according to Dixon (α = 0.02) and was therefore disregarded.

	Lab no.	**2**	**3**	**8**	**9**	**10**	**12**	
cBRP2	A	139.9	139.2	138.2	139.8	139.5	138.6	cp. Table 2a
	R	147.7	146.6	146.1	147.2	147.9	145.8	cp. Table 2b
	Mean	143.8	142.9	142.2	143.5	143.7	142.2	
Mean of means	143.0 ± 0.7		CV = 0.5%		(n = 6 labs)			
A: Ibio-numbers calculated using isoform factors deduced from ref [18]								
R: Ibio-numbers calculated using isoform factors deduced from ref [19]								

Table 2c: Mean of means. Note: Lab 11 (not shown) proved to be an outlier according to Grubbs (α = 0.05) and according to Dixon (α = 0.02) and was therefore disregarded.

Peak No.	**Amgen IU/µg poly-peptide**		**Lab 1**		**Lab 2**		**Lab 3**		**Lab 4**		**Lab 6**		**Lab 7**		**Lab 9**		**Lab 13**		**Lab 15**		**Lab 16**	
		Factor	pn	in	pn	in	pn	in	pn	in	pn	in	pn	in	pn	in	pn	in	pn	in	pn	in
1	50.3	0.50	0.1	0.1	0.0	0.0	0.5	0.3	0.0	0.0	0.3	0.2	0.0	0.0	0.1	0.1	0.0	0.0	0.2	0.1	0.1	0.1
2	70.6	0.71	1.1	0.8	0.4	0.3	1.5	1.1	1.0	0.7	1.3	0.9	1.1	0.8	0.7	0.5	0.6	0.4	1.1	0.8	1.0	0.7
3	96.6	0.97	4.8	4.6	5.8	5.6	4.9	4.7	4.2	4.1	4.9	4.7	4.5	4.3	2.7	2.6	4.3	4.2	4.8	4.6	4.6	4.4
4	170.3	1.70	16.7	28.4	16.8	28.6	16.9	28.8	16.1	27.4	17.4	29.6	17.5	29.8	17.4	29.6	16.6	28.3	17.0	29.0	16.9	28.8
5	255.8	2.56	26.8	68.6	26.6	68.0	26.4	67.5	27.5	70.3	27.3	69.8	26.9	68.8	28.8	73.7	27.5	70.3	26.4	67.5	27.5	70.3
6	258.4	2.58	29.8	77.0	29.7	76.7	29.6	76.5	30.2	78.0	29.5	76.2	29.2	75.5	30.0	77.5	29.8	77.0	29.7	76.7	29.7	76.7
7	258.7	2.59	18.9	48.9	18.8	48.6	18.4	47.6	19.4	50.2	17.9	46.3	18.8	48.6	18.5	47.9	18.9	48.9	18.6	48.1	18.1	46.8
8	205.8	2.06	1.8	3.7	1.9	3.9	1.8	3.7	1.6	3.3	1.5	3.1	2.0	4.1	1.8	3.7	2.3	4.7	2.0	4.1	1.8	3.7
Ibio_A_primary =			232.1		231.8		230.1		234.0		230.9		231.9		235.5		233.8		231.0		231.6	
Ibio_A_final =			139.2		139.1		138.1		140.4		138.5		139.2		141.3		140.3		138.6		139.0	
Mean Ibio_A = 139.4 ± 1.0							CV = 0.7%				(n = 10)											

Table 3a: BRP2 Ibio-number calculation and inter-laboratory precision. CZE data derived from Table 3c of Behr-Gross et al. 2007 [14] - Isoform distribution (in %) of BRP2 uncorrected for migration time, using isoform bioactivities published by Amgen [18].

Peak No.	**Roche IU/µg protein**		**Lab 1**		**Lab 2**		**Lab 3**		**Lab 4**		**Lab 6**		**Lab 7**		**Lab 9**		**Lab 13**		**Lab 15**		**Lab 16**	
	#)	Factor	pn	in	pn	in	pn	in	pn	in	pn	in	pn	in	pn	in	pn	in	pn	in	pn	in
1	19	0.19	0.1	0.0	0.0	0.0	0.5	0.1	0.0	0.0	0.3	0.1	0.0	0.0	0.1	0.0	0.0	0.0	0.2	0.0	0.1	0.0
2	40	0.40	1.1	0.4	0.4	0.2	1.5	0.6	1.0	0.4	1.3	0.5	1.1	0.4	0.7	0.3	0.6	0.2	1.1	0.4	1.0	0.4
3	75	0.75	4.8	3.6	5.8	4.4	4.9	3.7	4.2	3.2	4.9	3.7	4.5	3.4	2.7	2.0	4.3	3.2	4.8	3.6	4.6	3.5
4	150	1.50	16.7	25.1	16.8	25.2	16.9	25.4	16.1	24.2	17.4	26.1	17.5	26.3	17.4	26.1	16.6	24.9	17.0	25.5	16.9	25.4
5	200	2.00	26.8	53.6	26.6	53.2	26.4	52.8	27.5	55.0	27.3	54.6	26.9	53.8	28.8	57.6	27.5	55.0	26.4	52.8	27.5	55.0
6	280	2.80	29.8	83.4	29.7	83.2	29.6	82.9	30.2	84.6	29.5	82.6	29.2	81.8	30.0	84.0	29.8	83.4	29.7	83.2	29.7	83.2
7	400	4.00	18.9	75.6	18.8	75.2	18.4	73.6	19.4	77.6	17.9	71.6	18.8	75.2	18.5	74.0	18.9	75.6	18.6	74.4	18.1	72.4
8	205.8	2.06	1.8	3.7	1.9	3.9	1.8	3.7	1.6	3.3	1.5	3.1	2.0	4.1	1.8	3.7	2.3	4.7	2.0	4.1	1.8	3.7
Ibio_R_primary =			245.5		245.2		242.7		248.2		242.2		244.9		247.7		247.1		244.1		243.5	
Ibio_R_final =			147.3		147.1		145.6		148.9		145.3		147.0		148.6		148.3		146.4		146.1	
Mean Ibio_R = 147.1 ± 1.2					CV = 0.8%				(n = 10)													

Table 3b: Using isoform bioactivities published by Roche [19]. #) Specific activity of isoform peak no. 8 as published by Amgen [18] as the specific activity of this isoform of Roche [19] was not available.

	Lab no.	1	2	3	4	6	7	9	13	15	16	
BRP2	A	139.2	139.1	138.1	140.4	138.5	139.2	141.3	140.3	138.6	139.0	Cp. Table 3a
	R	147.3	147.1	145.6	148.9	145.3	147.0	148.6	148.3	146.4	146.1	Cp. Table 3b
	Mean	143.3	143.1	141.9	144.7	141.9	143.1	145.0	144.3	142.5	142.5	
Mean of means	143.2 ± 1.1	CV = 0.8%	(n = 10 labs)									

Table 3c: Mean of means. A: Ibio-numbers calculated using isoform factors deduced from ref [18], R: Ibio-numbers calculated using isoform factors deduced from ref [19].

Peak No.	Amgen IU/µg poly-peptide		Lab 1		Lab 2		Lab 3		Lab 4		Lab 5	
		Factor	pn	in	pn	in	pn	in	pn	in	pn	in
1	50.3	0.50	0.7	0.4	0.4	0.2	0.5	0.3	0.5	0.3	0.6	0.3
2	70.6	0.71	2.4	1.7	1.2	0.8	2.0	1.4	1.7	1.2	2.1	1.5
3	96.6	0.97	5.3	5.1	3.2	3.1	6.0	5.8	5.7	5.5	5.7	5.5
4	170.3	1.70	17.3	29.5	16.0	27.2	18.3	31.2	19.6	33.4	18.4	31.3
5	255.8	2.56	26.6	68.0	27.7	70.9	27.4	70.1	27.4	70.1	27.7	70.9
6	258.4	2.58	28.2	72.9	30.9	79.8	27.7	71.6	27.9	72.1	27.7	71.6
7	258.7	2.59	17.7	45.8	19.0	49.2	16.5	42.7	15.8	40.9	16.3	42.2
8	205.8	2.06	1.8	3.7	1.6	3.3	1.6	3.3	1.4	2.9	1.5	3.1
Ibio_A_primary =			227.0		234.5		226.3		226.3		226.3	
Ibio_A_final =			136.2		140.7		135.8		135.8		135.8	
Mean Ibio_A =	136.9 ± 2.2	CV = 1.6%	(n = 5)									
Mean Ibio_A =	135.9 ± 0.2	CV = 0.2%	(n = 4)									
Note: Lab 2 proved to be an outlier according to Grubbs (α = 0.01) and according to Dixon (α = 0.001) and was therefore disregarded.												

Table 4a: BRP3 Ibio-number calculation and inter-laboratory precision. CZE data derived from Table 7 of Burns et al. 2015 [15] - Isoform content of BRP3 (in %) uncorrected, using isoform bioactivities published by Amgen [18].

Peak No.	Roche IU/µg protein		Lab 1		Lab 2		Lab 3		Lab 4		Lab 5	
	#)	Factor	pn	in	pn	in	pn	in	pn	in	pn	in
1	19	0.19	0.7	0.1	0.4	0.1	0.5	0.1	0.5	0.1	0.6	0.1
2	40	0.40	2.4	1.0	1.2	0.5	2.0	0.8	1.7	0.7	2.1	0.8
3	75	0.75	5.3	4.0	3.2	2.4	6.0	4.5	5.7	4.3	5.7	4.3
4	150	1.50	17.3	26.0	16.0	24.0	18.3	27.5	19.6	29.4	18.4	27.6
5	200	2.00	26.6	53.2	27.7	55.4	27.4	54.8	27.4	54.8	27.7	55.4

6	280	2.80	28.2	79.0	30.9	86.5	27.7	77.6	27.9	78.1	27.7	77.6
7	400	4.00	17.7	70.8	19.0	76.0	16.5	66.0	15.8	63.2	16.3	65.2
8	205.8	2.06	1.8	3.7	1.6	3.3	1.6	3.3	1.4	2.9	1.5	3.1
Ibio_R_primary =			237.7		248.2		234.5		233.5		234.1	
Ibio_R_final =			142.6		148.9		140.7		140.1		140.4	
Mean Ibio_R = 142.5 ± 3.7 CV = 2.6% (n = 5)												
Mean Ibio_R = 141.0 ± 1.1 CV = 0.8% (n = 4)												
Note: Lab 2 proved to be an outlier according to Grubbs (α = 0.05) and according to Dixon (α = 0.05) and was therefore disregarded.												

Table 4b: Using isoform bioactivities published by Roche [19]. #) Specific activity of isoform peak no. 8 as published by Amgen [18] as the specific activity of this isoform of Roche [19] was not available.

BRP3	**Lab no.**	**1**	**3**	**4**	**5**	cp. Table 4a
	A	136.2	135.8	135.8	135.8	
	R	142.6	140.7	140.1	140.4	cp. Table 4b
	Mean	139.4	138.2	137.9	138.1	
Mean of means 138.4 ± 0.7 CV = 0.5% (n = 4 labs)						
Note: Lab 2 (not shown) proved to be an outlier according to Grubbs (α = 0.05) and according to Dixon (α = 0.01) and was therefore disregarded.						

Table 4c: Mean of means. A: Ibio-numbers calculated using isoform factors deduced from ref [18]. R: Ibio-numbers calculated using isoform factors deduced from ref [19].

Peak No.	**Amgen IU/µg poly-peptide**		**Lab 1**		**Lab 2**		**Lab 3**		**Lab 4**		**Lab 5**	
		Factor	pn	in	pn	in	pn	in	pn	in	pn	in
1	50.3	0.50	0.5	0.3	0.6	0.3	0.5	0.3	0.6	0.3	0.6	0.3
2	70.6	0.71	2.1	1.5	1.9	1.3	2.0	1.4	2.0	1.4	2.0	1.4
3	96.6	0.97	5.6	5.4	5.3	5.1	6.2	6.0	5.2	5.0	6.2	6.0
4	170.3	1.70	16.9	28.8	17.2	29.3	18.0	30.7	18.1	30.8	18.1	30.8
5	255.8	2.56	26.1	66.8	26.5	67.8	26.4	67.5	27.6	70.6	26.4	67.5
6	258.4	2.58	28.8	74.4	28.9	74.7	28.2	72.9	28.2	72.9	28.3	73.1
7	258.7	2.59	18.4	47.6	17.5	45.3	17.1	44.2	17.0	44.0	16.9	43.7
8	205.8	2.06	1.7	3.5	2.2	4.5	1.6	3.3	1.2	2.5	1.3	2.7
Ibio_primary =			228.2		228.3		226.2		227.5		225.6	
Ibio_final =			136.9		137.0		135.7		136.5		135.3	
Mean Ibio = 136.3 ± 0.7 CV = 0.5% (n = 5)												

Table 5a: cCRS Ibio-number calculation and inter-laboratory precision. CZE data derived from Table 6 of Burns et al. 2015 [15] - Isoform content of cCRS (in %) uncorrected, using isoform bioactivities published by Amgen [18].

Peak No.	Roche IU/µg protein		Lab 1		Lab 2		Lab 3		Lab 4		Lab 5	
	#)	Factor	pn	in	pn	in	pn	in	pn	in	pn	in
1	19	0.19	0.5	0.1	0.6	0.1	0.5	0.1	0.6	0.1	0.6	0.1
2	40	0.40	2.1	0.8	1.9	0.8	2.0	0.8	2.0	0.8	2.0	0.8
3	75	0.75	5.6	4.2	5.3	4.0	6.2	4.7	5.2	3.9	6.2	4.7
4	150	1.50	16.9	25.4	17.2	25.8	18.0	27.0	18.1	27.2	18.1	27.2
5	200	2.00	26.1	52.2	26.5	53.0	26.4	52.8	27.6	55.2	26.4	52.8
6	280	2.80	28.8	80.6	28.9	80.9	28.2	79.0	28.2	79.0	28.3	79.2
7	400	4.00	18.4	73.6	17.5	70.0	17.1	68.4	17.0	68.0	16.9	67.6
8	205.8	2.06	1.7	3.5	2.2	4.5	1.6	3.3	1.2	2.5	1.3	2.7
Ibio_primary =			240.4		239.1		236.0		236.6		235.0	
Ibio_final =			144.3		143.5		141.6		142.0		141.0	
Mean Ibio = 142.5 ± 1.4 CV = 0.9% (n = 5)												

Table 5b: Using isoform bioactivities published by Roche [19]. #) Specific activity of isoform peak no. 8 as published by Amgen [18] as the specific activity of this isoform of Roche [19] was not available.

	Lab no.	1	2	3	4	5	
cCRS	A	136.9	137.0	135.7	136.5	135.3	cp. Table 5a
	R	144.3	143.5	141.6	142.0	141.0	cp. Table 5b
	Mean	140.6	140.2	138.7	139.2	138.2	
Mean of means 139.4 ± 1.0 CV = 0.7% (n = 5 labs)							

Table 5c: Mean of means. A: Ibio-numbers calculated using isoform factors deduced from ref [18], R: Ibio-numbers calculated using isoform factors deduced from ref [19].

	Lab no.	2	3	8	9	10	11	
BRP1	A	136.3	134.9	133.3	135.2	135.1	134.0	cp. Table 1a
	R	138.9	137.2	135.6	138.3	138.0	135.8	cp. Table 1b
	Mean	137.6	136.1	134.5	136.7	136.5	134.9	
Mean of means 136.0 ± 1.2 CV = 0.9% (n = 6 labs)								
	Lab no.	2	3	8	9	10	12	
cBRP2	A	139.9	139.2	138.2	139.8	139.5	138.6	cp. Table 2a
	R	147.7	146.6	146.1	147.2	147.9	145.8	cp. Table 2b
(1)	Mean	143.8	142.9	142.2	143.5	143.7	142.2	
Mean of means 143.0 ± 0.7 CV = 0.5% (n = 6 labs)								

	Lab no.	1	2	3	4	6	7	9	13	15	16	
BRP2	A	139.2	139.1	138.1	140.4	138.5	139.2	141.3	140.3	138.6	139.0	cp. Table 3a
	R	147.3	147.1	145.6	148.9	145.3	147.0	148.6	148.3	146.4	146.1	cp. Table 3b

	Mean	143.3	143.1	141.9	144.7	141.9	143.1	145.0	144.3	142.5	142.5	
Mean of means	143.2 ± 1.1	CV = 0.8%	(n = 10 labs)									
	Lab no.	1	2	3	6	7	9	13	15	16		
cBRP3	A	137.0	137.0	136.1	137.2	138.9	136.7	137.0	135.6	137.1	cp. ref [6]	
	R	142.2	143.2	141.0	142.9	145.2	141.6	142.2	140.6	141.9	cp. ref [6]	
(2)	Mean	139.6	140.1	138.6	140.0	142.1	139.1	139.6	138.1	139.5		
Mean of means	139.6 ± 1.1	CV = 0.8%	(n = 9 labs)									
	Lab no.	1	3	4	5							
BRP3	A	136.2	135.8	135.8	135.8	cp. Table 4a						
	R	142.6	140.7	140.1	140.4	cp. Table 4b						
(3)	Mean	139.4	138.2	137.9	138.1							
Mean of means	138.4 ± 0.7	CV = 0.5%	(n = 4 labs)									
	Lab no.	1	2	3	4	5						
cCRS	A	136.9	137.0	135.7	136.5	135.3	cp. Table 5a					
	R	144.3	143.5	141.6	142.0	141.0	cp. Table 5b					
	Mean	140.6	140.2	138.7	139.2	138.2						
Mean of means	139.4 ± 1.0	CV = 0.7%	(n = 5 labs)									

(1) For cBRP2, lab 11 was an outlier according to Grubbs (α = 0.05) and according to Dixon (α = 0.02) and was therefore disregarded.

(2) For cBRP3, lab 4 was an outlier according to Grubbs (α = 0.01) and according to Dixon (α = 0.01) and was therefore disregarded.

(3) For BRP3, lab 2 was an outlier according to Grubbs (α = 0.05) and according to Dixon (α = 0.01) and was therefore disregarded.

Table 6: Ibio-numbers and inter-laboratory precision (Summary Table). A: Ibio-numbers calculated using isoform factors deduced from ref [18], R: Ibio-numbers calculated using isoform factors deduced from ref [19].

Data from		Ibio-number [IU/µg]	Stated bioactivity [ref] [IU/µg]	Accuracy against stated bioactivity [%]	Difference to stated bioactivity [%]	Remarks
BRP1	Table 1	136.0	130.0 [12]	104.7	+4.7	6 labs, study 2004 [13]
cBRP2	Table 2	143.0	130.0 [13]	110.0	+10.0	
BRP2	Table 3	143.2	130.0 [13]	110.2	+10.2	10 labs, study 2007 [14]
cBRP3	ref [6]	139.6	141.1 [14]	98.9	-1.1	
BRP3	Table 4	138.4	141.1 [14]	98.1	-1.9	5 labs, study 2015 [15]
cCRS	Table 5	139.4	141.1 #)	98.8	-1.2	

#) The stated bioactivity of BRP3 [14] was taken as reference value = 100%.

Table 7: Ibio-number accuracy determination of sample versus stated bioactivity (Summary Table).

Results

A reference electropherogram is shown in Figure 1.

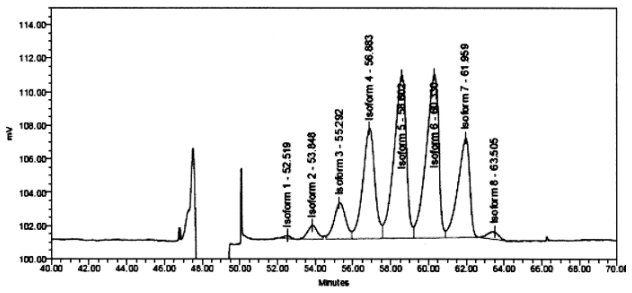

Figure 1: CZE electropherogram of EPO cBRP3, taken from the 2007 study [14] – with permission by EDQM.

Inter-laboratory precision

Ibio-number calculation for BRP1 from the CZE data published in the 2004 study [13] provided Ibio_BRP1 = 136.0 IU/µg ± 1.2 IU/µg, corresponding with an inter-laboratory precision of CV = 0.9% (n = 6 labs) (Table 1).

Ibio-number calculation for cBRP2 from the CZE data published in the 2004 study [13] provided Ibio_cBRP2 = 143.0 IU/µg ± 0.7 IU/µg, corresponding with an inter-laboratory precision of CV = 0.5% (n = 6 labs) (Table 2).

Ibio-number calculation for BRP2 from the CZE data published in the 2007 study [14] provided Ibio_BRP2 = 143.2 IU/µg ± 1.1 IU/µg, corresponding with an inter-laboratory precision of CV = 0.8% (n = 10 labs) (Table 3), confirming the data of the previous study [13].

Ibio-number calculation for cBRP3 from the CZE data published in the 2007 study [14] provided Ibio_cBRP3 = 139.6 IU/µg ± 1.1 IU/µg, corresponding with an inter-laboratory precision of CV = 0.8%; n = 9 labs) (for the corresponding Ibio-number calculation see ref [6]).

Ibio-number calculation for BRP3 from the CZE data published in the 2015 study [15] provided Ibio_BRP3 = 138.4 IU/µg ± 0.7 IU/µg, corresponding with an inter-laboratory precision of CV = 0.5%; n = 4 labs) (Table 4), confirming the data of the previous study [14].

Ibio-number calculation for cCRS from the CZE data published in the 2015 study [15] provided Ibio_cCRS = 139.4 IU/µg ± 1.0 IU/µg, corresponding with an inter-laboratory precision of CV = 0.7% (n = 5 labs) (Table 5).

In summary, the CZE data of the three collaborative studies [13-15] revealed for the Ibio-number assay an inter-laboratory precision of CV <1.0% each, ranging from CV = 0.5% (cBRP2, BRP3) to CV = 0.9% (BRP1) (Table 6).

Accuracy

The bioactivities calculated via the Ibio-number assay were compared with the stated potencies of the BRPs, which were regarded as the "true" potency values (= 100%, each), as summarized in Table 7.

The bioactivity calculated for BRP1 from the CZE data of the 2004 study [13] (Ibio_BRP1 = 136.0 IU/µg) differed from the stated bioactivity of BRP1 (130.0 IU/µg) by +4.7% (accuracy = 104.7%).

The bioactivity calculated for cBRP2 from the CZE data of the 2004 study [13] (Ibio_cBRP2 = 143.0 IU/µg) differed from the stated bioactivity of BRP2 (130.0 IU/µg) by +10.0% (accuracy = 110.0%).

The bioactivity calculated for BRP2 from the CZE data of the 2007 study [14] (Ibio_BRP2 = 143.2 IU/µg) differed from the stated bioactivity of BRP2 (130.0 IU/µg) by +10.2% (accuracy = 110.2%).

The bioactivity calculated for cBRP3 from the CZE data of the 2007 study [14] (Ibio_cBRP3 = 139.6 IU/µg) matched the stated bioactivity of BRP3 (141.1 IU/µg) with an accuracy of 98.9% (difference = -1.1%) [6].

The bioactivity calculated for BRP3 from the CZE data of the 2015 study [15] (Ibio_BRP3 = 138.4 IU/µg) matched the stated bioactivity of BRP3 (141.1 IU/µg) with an accuracy of 98.1% (difference = -1.9%).

The bioactivity calculated for cCRS from the CZE data of the 2015 study [15] (Ibio_cCRS = 139.4 IU/µg) matched the stated bioactivity of BRP3 (141.1 IU/µg) with an accuracy of 98.8% (difference = -1.2%).

Discussion

The data summarized herein may be regarded as a retrospective application of the Ibio-number assay to CZE data of three collaborative studies [13-15], dealing with EPO BRPs, with respect to "inter-laboratory precision" (same material, different labs, different studies) and "accuracy" (Ibio-number versus stated bioactivity set 100%).

Inter-laboratory precision

The high inter-laboratory precision of the Ibio-number assay has already been shown elsewhere for cBRP3 on the basis of the CZE data of the 2007 collaborative study [14] which provided CV = 0.8% (n = 9 labs) [6]. The inter-laboratory precision calculated from the CZE data of the 2004 [13] and the 2015 [15] collaborative studies were likewise high, resulting in CV <1.0% per study (Table 6). This precise set of data primarily relies on the precision and accuracy of CZE. But exactly for that reason, this data simultaneously reflects the bioactivity of the EPO samples in CZE in a likewise precise manner [6]. Thus, CZE of EPO samples provides a means for EPO bioactivity determination of previously unmet precision. The question was whether these precisely determined potency values are likewise accurate, which is discussed in the next section.

Accuracy

A separate retrospective analysis by the author of the CZE data of the various collaborative studies [13-15] via the I-number assay [10] has provided evidence that the bioactivities of EPO BRP1 and EPO BRP2 have been stated ~5% and ~10% too low, respectively [11]. And similar results have been obtained in the current study, relating the Ibio-numbers calculated for the (c)BRPs to the stated bioactivities of the corresponding BRPs.

As can be seen from summary Table 7, the potency calculated via the Ibio-number assay for EPO BRP1 (from the CZE data of the 2004 study [13]) did not fit to the stated potency of EPO BRP1 (difference = +4.7%).

Likewise, the potency calculated via the Ibio-number assay for cBRP2 (from the CZE data of the 2004 study [13]) did not fit to the stated potency of EPO BRP2 (difference = +10.0%).

Likewise, the potency calculated via the Ibio-number assay for BRP2 (from the CZE data of the 2007 study [14]) did not fit to the stated potency of EPO BRP2 (difference = +10.2%).

In contrast, the potency calculated via the Ibio-number assay for cBRP3 (from the CZE data of the 2007 study [14]) fitted well with the stated bioactivity of EPO BRP3 (difference = -1.1%).

Likewise, the potency calculated via the Ibio-number assay for BRP3 (from the CZE data of the 2015 study [15]) matched the stated bioactivity of EPO BRP3 (difference = -1.9%).

And likewise, the potency calculated via the Ibio-number assay for cCRS (from the CZE data of the 2015 study [15]) matched the stated bioactivity of EPO BRP3 (difference = -1.2%).

Why was there such a clear fitting for calculated (c) BRP3 versus stated BRP3 and calculated cCRS versus stated BRP3 and such a clear mismatch for calculated BRP1 versus stated BRP1 as well as calculated (c)BRP2 versus stated BRP2?

An explanation of these mismatches for EPO BRP1 and EPO BRP2 has been given by the author on the basis of the I-number assay elsewhere [11], and the same explanation applies to the Ibio-number assay of the current study. In brief, the 2004 study [13] has neglected that the differences in the isoform composition of EPO BRP1 and cBRP2, as revealed in CZE, necessarily must have changed the bioactivity of the product when changing from BRP1 to BRP2. Yet, the potency of BRP2 has been assigned to 32,500 IU/vial, i.e. identical to BRP1. This identical potency assignment did not consider the fact that the percent shares of isoforms 3 and 7 of BRP1 and cBRP2 had changed (IF3BRP1 = 6.14%, IF3cBRP2 = 4.68%; IF7BRP1 = 14.51%, IF7cBRP2 = 18.09%; cp. Table 3a and Table 3b of ref [13]), resulting in different Ibio-numbers (Ibio_BRP1 = 136.0 IU/µg, Table 1; Ibio_cBRP2 = 143.0 IU/µg, Table 2). Hence, the Ph. Eur. specification of isoforms 3 and 7 had to be changed (Ph. Eur. 2002 [23]: IF3 = 5-20%, IF7 = 0-20%; Ph. Eur. 2008 [24]: IF3 = 1-20%; IF7 = 5-25%). The lower content (pn) of isoform 3 concomitant with the higher content (pn) of isoform 7 in BRP2 is clearly reflected in the Ibio-numbers which increased from Ibio_BRP1 = 136.0 IU/µg to Ibio_cBRP2 = 143.0 IU/µg (an increase of ~5.0%), however was not expressed in the assigned bioactivities of 32,500 IU/vial, each.

Thus, erythropoietin medicinal products that have been calibrated against erythropoietin reference preparation batch 1 or batch 2 have been subject to the same error which was, however, within the error of the mouse bioassay and therefore not crucial. The validity of the potencies achieved upon calibration against EPO BRP1 or EPO BRP2 was also ensured by the broad range allowed by Ph. Eur. for potency determination ("The estimated potency is not less than 80 per cent and not more than 125 per cent of the stated potency. The confidence limits of the estimated potency (P = 0.95) are not less than 64 per cent and not more than 156 per cent of the stated potency" [7,23,24]).

No CZE data were provided in the 2015 study dedicated to establish EPO BRP batch 4 [16], as this batch was limited to serve as a reference for the bioassay in mice and not intended as a reference for physicochemical assays. EPO BRP batch 4 and EPO cCRS were prepared from the same material. Hence, their bioactivities should be identical (i.e., 139.4 IU/µg), as calculated for Ibio_cCRS (Table 5).

EPO BRP batch 4 has an assigned potency of 13 000 IU/vial and contains around 100 µg of EPO per vial [16], equivalent to a potency of 130.0 IU/µg, which in fact differs from the calculated potency (Ibio_cCRS = 139.4 IU/µg) by ~7%, which is further addressed below.

The WHO 3rd IS for erythropoietin, recombinant, for bioassay (11/170) is not intended as a reference standard for CZE either; therefore, again no CZE data have been published for this material. Noteworthy, "the third IS for erythropoietin is no longer a mixture of alpha and beta erythropoietin but contains only epoetin alpha" [25]. Hence, Ibio-number calculation is solely based on the isoform bioactivities of epoetin alfa, which is marketed in the USA by Amgen (for general anemia) and by Johnson and Johnson (for anemias of cancer) [25]. In fact, an epoetin alfa material from Johnson & Johnson Ortho Biotec was retrospectively used by the author to calculate the Ibio-number that could reflect the potency of the 3rd IS, namely Centocor's epoetin alfa secondary standard, the CZE data of which have been made available to the public [22]. However, it is unclear (at least to the author) whether the EPO material used to prepare the 3rd IS was in fact from Johnson& Johnson. Nevertheless, the potency of this epoetin alfa secondary standard from Centocor (respectively Johnson & Johnson) was calculated by the author via the Ibio-number assay, resulting in Ibio_α = 142.3 IU/µg [6]. This potency matched the declared potency of the 3rd IS (set 100%) with an accuracy of 94.9% (difference = -5.1%).

The reason for this difference may be due to the chosen manufacturer or due to different batches (of the same manufacturer) or due to the lack of precision of the mouse bioassay or each of these (and the same arguments hold true for EPO BRP4 batch 4, see above). In fact, the mouse bioassay is highly inaccurate (CV ≈ 25% [1,2], ≈ 20% [3,4]; uncertainty 15-30% [5] as stated by Zimmermann et al. [3]), and lack of batch-to-batch consistency of biologics or biosimilars will remain an issue for each manufacturer. For example, the bioactivity determined in normocythaemic mice of 17 epoetin beta 'validation batches' from Roche, which were of the medium range of Roche's well established NeoRecormon® production, covered a range of 187.0–241.4 IU/µg (annotation: "IU/µg protein") [3], counting for a difference of these two values of ~25%.

This indicates the major problem in assigning what may be regarded as the "true" potency value of erythropoietin: It will hardly be possible to compare the results of the highly precise and accurate Ibio-number assay with potencies measured via the most variable mouse bioassay in a reliable way – unless the bioassay data are gathered in an elaborate collaborative study – as the mouse bioassay "may lead to considerable ranges in activity for both biosimilar and reference product" [26]. Hence, the physicochemical data of EPO potency determination presented herein my support the recently diagnosed "paradigm shift in the European Union", foreseeing "biosimilars entering the clinic without animal studies" [26].

In this regard, it is interesting to know – and this is therefore repeated here – that "in the EU, a new Directive on the protection of animals used for scientific purposes was issued in 2010, which updates and replaces the 1986 Directive 86/609/EEC. The aim of the new Directive is to strengthen legislation, and improve the welfare of those animals still needed to be used, as well as to firmly anchor the principle of the "Three Rs," to Replace, Reduce and Refine the use of animals, in EU legislation. Directive 2010/63/EU has taken full effect from 1 January 2013. According to this Directive, the use of animals for scientific or educational purposes should only be considered where a non-animal alternative is unavailable (preamble 12) and Member States shall ensure that, wherever possible, a scientifically satisfactory method or testing strategy, not entailing the use of live animals, is used instead (Article 4.1)" [26].

Perhaps the Ibio-number assay for EPO medicinal products may, like the I-number assay [11,27], turn out to be such an alternative which, on the long term, could save thousands of mice and a lot of money, as well.

Noteworthy, the bioactivities of the EPO samples retrospectively calculated by the author via the Ibio-number assay herein and elsewhere [6] and the I-number assay [11,27] were very comparable – with differences between the two assays of <2.0%, each time, and a mean difference of 1.2%. This closeness of the results is, however, not surprising as both assays are based on the same CZE data.

This quasi-identity of the results of the I-number and the Ibio-number assay shows that it is not necessary to choose the Ibio-number assay for accurate potency determination and to know the bioactivity of the individual EPO isoforms respectively the multiplication factors (cp. Tables 1-5), the determination of which is very laborious, time consuming and costly and needs sacrifice of hundreds of mice. The same results, with the same precision and accuracy, can be achieved using the "simple" I-number assay [11,27].

The advantage of the I-number assay is that this assay can be used without knowing the bioactivities of the individual EPO isoforms, which makes this assay readily applicable for any laboratory and will most likely render it first choice at least during product development and scale-up production. The advantage of the Ibio-number assay is that – once the bioactivities of the EPO isoforms (respectively the multiplication factors) are known – this assay allows to directly calculate the bioactivity of the EPO samples, whereas the I-number assay requires measurement against an EPO reference standard of known bioactivity, in order to allow calculation of the bioactivity of the sample. But this is no disadvantage, as the EPO samples should anyway routinely be measured against a reference substance (currently "Erythropoietin for physicochemical tests CRS batch 1" [15]), which should allow to precisely and accurately monitor the bioactivity and the quality of the EPO medicinal products via the I-number and/or Ibio-number assay.

Conclusion

The data presented in this retrospective analysis by the author of the CZE data of various (c)BRPs and the cCRS has shown that the Ibio-number assay enables assessment of the potency of EPO reference preparations with high precision and accuracy, paralleling and confirming the results obtained with the I-number assay [11]. Thus, the very broad criteria for EPO identification via CZE according to the Ph. Eur. [7] (which is based on broad ranges defined for the various EPO isoforms) could be replaced by a single and quite narrow Ibio-number range, which would provide a significant increase in assay precision and accuracy and hence in drug safety. Moreover, the Ibio-number assay could be a candidate physicochemical assay to replace the mouse bioassay in the quality control of EPO batch release.

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