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Discussion paper: Transition from biological to chemical assay for quality assurance of medicinal substances (APIs) and formulated preparations

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Introduction

The transition from a biological approach , using biological assay methods reporting in International Units (IU) to chemical approach, using physico-chemical assay methods, reporting in SI units, is an evolutionary step, based to an increased understanding that links structure with function, and made possible by the continuing development and increasing utility of physico-chemical methods such as High Performance Liquid Chromatography (HLPC). The process has, for example, been completed for drugs such as steroids, thyroid drugs and adrenergic agents, and largely completed for antibiotics. The transition has also been made in a number of peptide and small protein drugs.

The implications of such a transition may be complicated by considerations of labeling and dose regimens. The question of how dosage of any particular medicine is expressed and hence the manner in which strength is stated on the product label requires broad consultation. There would need to be an international or, at least, regional consensus among all stakeholders including: manufacturers, regulatory and pharmacopoeial authorities, and users (clinicians, pharmacists, other health professionals, providers, patients). Any agreed transition from dosage/labelling in IU to dosage/labelling in weight would need to be very carefully planned and managed (timescale, information, education, co-ordination etc) and would need involvement of all above stakeholders.

Transition from biological assay to chemical assay

Appropriate scientific evidence must be available to justify placing reliance on chemical assay. The amount and type of evidence required may depend on the purpose of the assay (product characterization, routine quality control, pharmacopoeial compliance etc). Such transitions usually proceed gradually as evidence is acquired and confidence is built. As a first stage, a particular manufacturer may provide sufficient in-house data on his product to a regulatory authority to permit the company to switch to chemical assay for in-process control and/or product release. At a later stage, when data are available from several manufacturers and/or has been published and collaborative work has been carried out to establish a pharmacopoeial chemical reference substance, the switch may be made to the assay method specified in the relevant pharmacopoeia. Once this is done, the content limits in a pharmacopoeial monograph for a substance should be expressed in appropriate terms. For example in the case of synthetic peptides this may be percentage by weight, calculated with reference to the anhydrous/acetic acid-free (as appropriate) substance. On the other hand, for biosynthetic proteins, the content limits may be expressed in mols of protein, determined by a technique such as amino-acid analysis. In all cases, an equivalence statement should normally be included to permit the continued labelling of the corresponding formulated preparations in IU.

[For example, the European Pharmacopoeia monograph for Oxytocin has an HPLC assay with limits of 93.0 to 102.0 per cent of the peptide $C_{43}H_{66}N_{12}O_{12}S_2$, calculated with reference to the anhydrous, acetic acid-free substance and states "By convention, for the purposes of labelling oxytocin preparations, 1mg of oxytocin peptide ($C_{43}H_{66}N_{12}O_{12}S_2$) is equivalent to 600 IU of biological activity."]

It should be understood that the activity, previously regarded as a variable and defined by the actual WHO International Standard (reference preparation), is now considered an intrinsic property of the molecule. Thus, for oxytocin the assumption is that 1 mg of pure oxytocin will always be 600IU.

The purpose of the assay in a pharmacopoeial monograph for a formulated preparation is to determine whether the content of active ingredient is within acceptable limits of the declared content (labelled claim). Thus, for a chemical assay, the *analyst* requires the label to indicate the content in terms of weight of the substance. Until and unless there is a stakeholder consensus on changing dosage from IU, the *users* of the preparation (clinician, patient etc) will still require the strength to be declared in IU.

[For example, the British Pharmacopoeia monograph for Oxytocin Injection has an HPLC assay with limits for the content of oxytocin, $C_{43}H_{66}N_{12}O_{12}S_2$ of 90.0 to 110.0 % of the stated amount of the peptide and a Labelling statement: "The strength is stated as the number of IU (Units) per ml. The label also states the equivalent number of micrograms of oxytocin per ml." Note: the "label" in this context includes the package and/or leaflet.]

It should be noted that, even when reliance is placed on chemical assay for pharmacopoeial compliance testing of a substance and of its formulated product, biological assay may still be needed for other quality assurance purposes. Given that the same proteins can be differently produced by different manufacturers and that their structure also can be influenced by excipient and manufacturing process, there may be grounds to require for example that, during developmental stages, new products have the expected biological activities.

For example, the Ph Eur monograph for Somatropin states : *"during the course of product development, it must be demonstrated that the manufacturing process produces a product having a biological activity of not less than 2.5 IU/mg, using a validated bioassay based on growth promotion and approved by the competent authority"*

Chemical Reference substances As noted above, in switching from a biological to a chemical assay, a pharmacopoeia establishes a chemical reference substance (CRS) suitable for assay purposes. In contrast to the situation with biological reference preparations, pharmacopoeial CRS are not established by calibration against a global standard. Each pharmacopoeial CRS is thus a primary reference substance. An

International Chemical Reference Substance (ICRS) established by the WHO Collaborating Centre in Sweden is therefore, usually, one of several primary reference substances, rather than *the* primary reference substance.
http://www.who.int/medicines/publications/pharmprep/TRS943_PharmPrep.pdf

ICRS are established when needed to support a monograph in *The International Pharmacopoeia*. In relation to the examples given below, it should be noted that a monograph for Oxytocin is in preparation and the development of full monograph(s) for insulins has been proposed (the current entry in the Ph Int for Insulin cross refers to use of "methods described in other pharmacopoeias, such as those of China, Europe, Japan, UK and USA."). No proposals exist, at present, for Ph Int monographs for calcitonin (salmon) and somatropin (these substances are not included in current (15^{th}) Essential Medicines List).

Biological Reference Preparations/ International Standards

When the biological assay is no longer required by Member States for any purpose, the International Standard will strictly no longer be needed and could be withdrawn. However, careful consideration needs to be given to those cases where it is considered necessary to retain dosage and therefore product labelling in International Units as the "unit has no existence other than in relation to the reference preparation that defines it".

http://www.who.int/biologicals/expert_committee/TRS932CVR%20with%20full%20Texts.pdf

A number of possible approaches may be envisaged in such cases in order to enable the current dosage/labelling convention to be maintained:

1. Retain the existing IS, that is, a physical standard as an "archival IS" for as long as possible to provide definition of IU to support the "by convention equivalence" statements (as in eg Ph Eur monographs). Once the stock of the "archival IS" is depleted, or if there is evidence that the reference material is no longer fit for purpose, and if approaches 2 or 3 (below) are not considered acceptable, approach 1 would require consideration of establishing a replacement "archival IS" for any substances that, at that time, still required dosage and labelling in units of biological activity.

2. Establish a "virtual IS" to support the "by convention equivalence" statements (as in eg Ph Eur monographs). This would be an official WHO statement explaining that the conventional equivalence was established in relation to the IU as defined by the [ ] IS, established in …and withdrawn in … and that this historical definition of IU may still be used in order to maintain the current labelling convention of expressing dosage in IU.

3. Provide an official WHO statement explaining that the conventional equivalence was established in relation to the IU as defined by the [ ] IS, established in …and withdrawn in … and indicating that, while the IU no longer exists, the equivalence may be used in order to maintain the current convention of expressing dosage in Units of biological activity (where Unit is equivalent to the former IU) .

Recent history of compounds moving into/through the transition phase

Comparison of the four exemplars (Table) reveals that a consistent approach has not been taken. In the case of somatropin (recombinant growth hormone), a two stage international harmonization process was undertaken, demonstrating first, that the switch to physico-chemical methods was scientifically justified, and subsequently, establishing a WHO IS value assigned in both IU and SI. The process was transparent and supported by published data. For Calcitonin, SI and IU are formally assigned to the WHO IS on the basis of a collaborative study. For insulin and oxytocin the WHO IS does not have an SI value assigned, and for these two hormones the mg is traceable to regional or national standards with a separated mg value assignment. In the case of Insulin the agreed "by convention" potency was supported by published collaborative studies at both WHO and local level, whereas in the case of oxytocin these by-convention agreed values are made on the basis of data that have not been available through WHO.

Table: Four recent examples of products moving into/through the transition phase

Substance	***Consensus potency***	***Area of application***	***Dosing***	***Process***	***Standards***	***Current stock issue (/year)***	***Stucture***	***Production***
Human Insulin	28.82 IU/mg 26.6 USP U/mg	European Pharmacopoeia USP	IU USP U	International Collaborative study (IU) / local SI value assignment	WHO IS (IU), 83/500 Local standards (SI)	5-10 per year 1257 remaining	Covalent heterodimeric peptide, 51 aa, 6kD approx	Biosynthetic (recombinant)
Rec Growth Hormone (somatropin)	3.0 IU/mg 8.63 USP Somatropin U/2.88 mg	International USP	mg/IU mg	International Collaborative study	WHO IS, formally assigned in both SI and IU, 98/574	250 per year (includes immunoassay use) 6932 remaining	Monomeric globular protein, 191aa, mol wt = 22kD	Biosynthetic (recombinant)
Calcitonin	6000 IU/mg	European Pharmacopoeia No USP monograph	IU	International Collaborative study	WHO IS, formally assigned in both SI and IU, 98/586	5-10 per year 3408 remaining	Linear peptide, 32 aa	Synthetic or biosynthetic (recombinant)
Oxytocin	600 IU/mg No intrinsic potency, standard defined in units >400USO U/mg	European Pharmacopoeia USP	IU USP U	Limited local collaborative study	WHO IS (IU), 76/575 Local standards (SI)	Small, 0-5 ampoules/year 1427 remaining	Cyclic nonapeptide	Synthetic

Expression of dosage and labelling of product strength

The strength of a formulated preparation has to be stated in the same terms as are used for the dosage. Any proposal to change to the way the dose of a medicine is expressed needs very careful consideration and consultation of all those affected. Issues that need to be addressed include patient safety (the potential for medication errors), patient compliance and the burden/expense to stakeholders. A range of factors concerning the use of the medicine need to be taken into account including the disease/condition that it is used to treat/prevent, how widely and by whom it is prescribed and administered.

Considerations for the four examples

1.Somatropin

Doses are already expressed in weight (mg/µg) of somatropin; products are labelled in weight (with IU in parenthesis in some regions). Treatment of growth disorders with somatropin by specialist physicians. Not high/wide usage - mainly in developed countries. Change to dose expression was made when switched from human pituitary growth hormone (HGH)to rDNA somatropin.

2.Calcitonin (salmon)

Doses are expressed in IU; products are labelled in IU and the weight of peptide in µg is also provided for purposes of analysis. Treatment of bone disorders with calcitonin (salmon) mainly by specialist physicians. Not high/wide usage - mainly in developed countries. *May be* relatively safe/easy to change to expressing dose by weight.

3.Oxytocin

Doses are expressed in IU; products are labelled in IU and the weight of peptide in µg is also provided for purposes of analysis. Used in induction of labour and prevention/treatment of postpartum haemorrhage by a range of health professionals and health providers (eg mid-wives).
High usage - WHO actively promoting use by "skilled attendants" as part of "Making Pregnancy Safer" http://www.who.int/making_pregnancy_safer/en/ (Millennium Development Goals for maternal and child health). Used by a wide variety of health providers including in emergency situations. A change to expressing dose by weight likely to be unwise/difficult.

4.Insulins

Doses are expressed in IU (or, in some countries, Units); products are labelled in IU (or, in some countries, Units). Treatment of diabetes with insulin by specialist physicians and general practitioners. Insulin is a very special case and there may be compelling reasons why a transition from IU to expressing dose by weight should not be contemplated for the foreseeable future:

- Injected by patients themselves (including children) or by carers on daily basis
- Dose varies and needs to be adjusted by patients/carers
- Dosage critical (hyper- and hypoglycaemia)
- Range of specially calibrated administration devices (eg syringes, pens)
- Wide range of preparations (different types of insulin and formulations with different durations of action)
- Very high level of usage (in developed countries)
- High but inadequate level of usage (in developing countries)

Diabetes is currently the focus of special WHO attention in collaboration with partners such as IDF http://www.who.int/diabetes/en/ Diabetes awareness/prevention and access to treatment are key issues for The Diabetes Action Now Programme. Access to and affordability of insulin is a serious problem in developing countries.

It would seem advisable to take the same approach for all insulins - porcine, bovine, human and analogues (eg insulin-lispro).

Request to the Expert Committee on Biological Standardization and the Expert Committee on Specifications for Pharmacuetical Preparations

Both Expert Committees, at their meetings in 2006, requested that WHO should issue some guidance on the use of its standards in this area -see WHO TRS 943, WHO 2007 http://www.who.int/medicines/publications/pharmprep/TRS943_PharmPrep.pdf.

The current discussion paper is provided to stimulate further comment from both Expert Committees and to assist the Secretariat in developing detailed plans to provide such guidance. A number of factors should be kept in mind as an action plan is developed:
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- For at least one of these molecules (oxytocin) the situation described above is regional rather than global. The WHO IS may still be used in its original global context in other areas of the world.
- The "by-convention" values accepted for all of these molecules have been in place and effective now for many years, with licensed products depending on them, and it is unlikely that any change arising from a new collaborative study, would be considered acceptable.
- Resource is limited both in the WHO collaborating laboratory, and in potential bioassay laboratories. The assays that the WHO standards support are very rarely carried out.
- A number of other WHO standards are in preparation which may be similarly dual-defined (eg Parathyroid hormone 1-34 and 1-84).
- The issue may be anticipated for other products, especially small proteins such as cytokines.
- Some biotherapeutics may be directly expressed as SI unit (for example, all monoclonal antibodies are expressed as mg) after their biological activity has been fully characterized.

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