PEGylated proteins: evaluation of their safety in the absence of definitive metabolism studies.

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Running Title: The ADME and toxicology of PEG associated with biologicals.

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Number of text pages: 28
Number of table: 2
Number of figure: 2
Number of references: 36
Number of words in the Abstract: 165
Number of words in the Introduction: 496
Number of words in the Review Section: 4162

Abbreviations

PEG: Polyethylene Glycol
kDa: Kilo Daltons
EPAR: European Public Assessment Report
Abstract.

During the development of any PEGylated protein or peptide toxicology in relevant species will be conducted prior to human exposure. Normally comprehensive metabolism data accompany the toxicity studies for a small molecule. We have examined if such studies would be relevant in the safety assessment of PEGylated material. Literature data indicate that the PEG associated with a biological molecule should provide no extra concern as the exposure : toxicity relationship of PEG in animals and human has been thoroughly investigated and metabolism / excretion of PEG is well understood. Based on the comparisons of PEG exposure from PEGylated biological products and the exposures of PEG associated with toxicity in human the therapeutic index is large (approximately 600 fold or greater). Therefore, assuming toxicological evaluation of a biological molecule of interest is complete and satisfactory therapeutic windows are achieved the data contained in this review indicate that the PEG associated with a protein or other biological molecule does not represent an additional un-quantified risk to humans.
Introduction.

The conjugation of small proteins, peptides and oligonucleotides with polyethylene glycol (PEG), or PEGylation, has become an increasingly common method of improving the half-life of biological products mainly through reducing the urinary excretion of the molecule (Yang et al., 2004) but also by reducing the enzymic degradation due to the increased steric bulk (Veronese and Pasut, 2005). In addition PEGylated biological products often exhibit a reduced affinity for the target receptor compared to the native pre-cursor. This reduced affinity can lead to a lower clearance by target-mediated clearance mechanisms. Finally, the addition of the PEG moiety can have beneficial effects on the immunological profile of a molecule by reducing the ability of the compound to raise antibodies in human (Mehvar, 2000).

PEG is a polymer made up of identical ethylene glycol subunits. PEGs have a descriptor associated with them that represents the mean molecular weight of the molecule (i.e., PEG200 has a molecular weight of 200) (Smyth et al., 1955). The PEG molecules conjugated to proteins can also have the terminal hydroxyl group capped with a methyl group (Molineux, 2003). The structures of these PEGs are detailed in Figure 1. Higher molecular weight PEGs can have some degree of branching. The PEGs used to conjugate biologicals are polydispersed in nature (i.e., have a range of molecular weights) and this can lead to a range of drug molecules with potentially subtly different biological properties. The impact of polydispersity must be considered when dealing with these conjugated biological agents (Veronese and Pasut, 2005).
In order to couple the PEG to the protein, peptide or oligonucleotide, the PEG (generally monomethoxy PEG) is first activated. Several methods can be used to achieve this activation and coupling including; cyanuric chloride, 1,1'-carbonyldiimidazole, phenylchloroformate or succidinimidyl active ester (Delgado et al., 1992; Mehvar, 2000). The mechanisms by which PEGs are coupled to proteins has also been discussed more recently by (Veronese and Pasut, 2005).

Toxicology studies with the PEGylated proteins are normally carried out before studies in humans and these provide excellent evidence of the safety of these PEG-containing molecules. Such safety studies, when performed on small organic molecules are normally accompanied with corresponding metabolism studies exploring the biological fate of the molecule in the various toxicology species and human. These studies are very difficult to conduct on the PEGylated material and this leaves a possible concern about the PEGylated portion of the molecule and its impact on human safety. In their non-conjugated form, polyethylene glycols are widely used as excipients for a large variety of drugs and are also regularly used in children for the treatment of paediatric constipation or for colonoscopy (Pashankar et al., 2003; Bell and Wall, 2004; Pashankar et al., 2004).

The purpose of this review is to consider the toxicology (animal and human), metabolism, excretion and exposures of humans to PEG and to evaluate the safety of the PEG on PEGylated proteins to those exposed to these biological products. The feasibility of conducting A.D.M.E. studies will also be considered as well as any value they would bring to the risk assessment.
Toxicology of polyethylene glycol in animals

Acute, short and long term toxicology studies with polyethylene glycols (PEGs) administered by the oral, intra-peritoneal and intravenous routes in a wide range of animal species have been carried out with PEGs with molecular weights of up to 10,000. The toxicity of PEG has been thoroughly reviewed (Fruijtier-Polloth, 2005), in light of this a brief summary of the toxicology of PEG is given below. Following acute administration the LD_{50} values of PEG are generally in the region of 10g/kg or higher. The clinical signs associated with this acute exposure include jumping, tremors, convulsions, piloerections and dyspnoea. Post mortem examination showed pulmonary hyperaemia and oedema (Fruijtier-Polloth, 2005). Acute poisoning in rabbits suggest that poisoning occurs at plasma concentrations in excess of 30-70mM (Herold et al., 1989).

In chronic oral toxicology studies in the rat PEG1500 (0.06g/kg/day) and PEG4000 (0.02g/kg/day) did not cause any significant adverse effects following 2 years administration (Smyth et al., 1955). When PEG1540 and 4000 were administered as part of the diet (4%) to rats, no effects were observed (Smyth et al., 1955). Experiment with PEG400 showed no effect at doses of 2% in diet, with higher doses showing non-specific effects on growth and cloudy swellings in the liver (Smyth et al., 1955). These data indicate that following chronic administration to the rat PEG is not a significant toxicological concern.

In chronic toxicology studies in non-rodent species there were no adverse events in dogs that received PEGs ranging from 400-4000 molecular weight (2% in diet) for a year (Smyth et al., 1955). In the monkey PEG200 caused pathological lesions during a
13 week study in the kidney at an oral dose of 2.2-4.4g/kg. The renal lesions consisted of intra tubular deposition of oxalate crystals in the renal cortex but were not associated with other clinical or pathological findings (Prentice and Majeed, 1978).

Adverse effects have also been observed in monkeys treated by intravenous infusion (1ml/h) for up to one month with 60% PEG400 in water (approximately 3g/kg/day). At this dose the monkeys had reduced appetite, a greasy texture of their lower extremities, edema of their genitalia and legs, and deteriorating infusion sites (Working et al., 1971; Frujitier-Polloth, 2005). These data indicate the excellent safety profile of PEG when administered chronically to non rodent species.

No adverse reproductive or teratogenic effects are reported with PEGs. Polyethylene glycols are neither mutagenic nor carcinogenic (Working et al., 1971; Frujitier-Polloth, 2005).

**Toxicology of PEGylated molecules in animals.**

Pre-clinical toxicology studies performed with pegylated proteins have also not revealed any PEG-specific toxic findings. For example, with peginterferon, the toxicity profile was evaluated in cynomolgus monkeys dosed subcutaneously for 4 weeks either twice weekly (dose up to 562.5 µg/kg) or daily (doses up to 600 µg/kg) and for 13 weeks administered twice weekly (doses up to 150 µg/kg). Peginterferon was well tolerated. The characteristic pattern of interferon alpha toxicity was observed with peginterferon. These effects included suppressive effects on the haematopoietic system and increases in liver enzymes (Pegasys® EPAR).
With Peg-Intron®, repeated dose toxicity studies were performed in cynomologous monkeys using subcutaneous doses administered every other day for one month. Important findings included decreases in all types of blood cells, serum proteins, calcium phosphorus and potassium. The findings observed in Peg-Intron®-dosed monkeys were similar to those produced by IntronA®. There were no unique toxicity due to the pegylation. Greater incidence and/or severity of the findings were noted in the high-dosed monkeys compared to IntronA®. This is in accordance with the prolonged exposure and higher AUC values obtained using Peg-Intron® (Peg-Intron®, EPAR).

Treatments with pegylated proteins such as peginterferon alpha-2a (40kDa; Pegasys®) and pegylated asparaginase do not reveal any specific adverse events linked to the PEG moiety (Schwarz et al., 2003); (Avramis et al., 2002).

Overall, the acute or chronic administration of PEG with a range of molecular weights by a range of routes has not led to any major toxicities and signs of toxicity that do occur are only apparent at high dose. In light of this information PEG can be considered to have a toxicological profile of very low concern in animals.

**Clearance of polyethylene glycol by metabolism.**

The metabolism of PEG has been investigated in animals and in humans. A summary of the results is presented in Table 1, along with data on the excretion of PEG.

**Metabolism of PEG.**
The metabolism of PEG itself is simple and involves the oxidation of the alcohol groups present on the PEG to a carboxylic acid. For example, the diacid and hydroxyl acid metabolites of PEG have been observed in the plasma and urine of burn patients and rabbits and in the bile of cats (Hunt et al., 1982; Friman et al., 1990; Friman et al., 1993). In the isolated guinea pig liver and in rat/guinea pig in vitro liver preparations PEG has been shown to be sulphated (Roy et al., 1987; Roy et al., 1988). Evidence from studies with PEG400 indicate that ethylene glycol is not formed as a metabolite of PEG in humans (Schaffer et al., 1950). Minor amounts of oxalic acid are liberated following the metabolism of PEG (Fruijtier-Polloth, 2005).

The phase 1 metabolism of PEG in mammalian systems is mediated by alcohol dehydrogenase (Herold et al., 1989). P450’s may also play a role in the oxidation of PEG (Veronese and Pasut, 2005) although the evidence for this is not clear. Also PEG has been shown to be metabolised by sulphatransferases (Roy et al., 1987; Roy et al., 1988). Whilst there is evidence that PEG can be metabolised to a series of phase 1 and phase 2 metabolites the toxicology data presented above indicates that these metabolites are of very little toxicological concern. However, metabolism of PEG to the acid metabolite/s has been implicated in the acidosis and hypercalcemia observed in patients following overdose (Bruns et al., 1982). It is clear that these metabolites can be formed in multiple toxicology species and that the phase 1 metabolites are seen in animals and human. These data indicate that human and animals will be exposed to similar metabolites following administration of PEG.

**Impact of molecular weight on PEG metabolic clearance.**
Excretion balance studies have been carried out in humans with PEG. Assuming that any PEG that has not been recovered intact in these studies has been metabolised prior to secretion, it is apparent that metabolic clearance of PEG decreases markedly as molecular weight increases. For PEG400 up to 25% of the dose may be metabolised in human (Schaffer et al., 1950), similar results are also seen in the rabbit (Schaffer et al., 1950). For PEG1000 and 6000 a maximum of 15% and 4% of the doses is cleared by metabolism, respectively (Schaffer et al., 1950). The human result on PEG1000 is supported by cat data with PEG900 that indicates that 75% of the biliary eliminated material (biliary elimination is likely to be a minor clearance pathway, see below) was unchanged, the rest representing oxidised metabolites (Friman et al., 1993).

Sulphation in the rat and guinea pig also shows a molecular weight dependency with PEG200 showing the highest rate and PEG6000 showing no detectable turn over (PEG200, 400, 1000 and 6000 investigated in the isolated perfused guinea pig liver) (Roy et al., 1987; Roy et al., 1988).

Overall the metabolic pattern of PEG in vivo is simple and involves the oxidation of the alcohol groups to carboxylic acids. This is likely to be mediated by alcohol dehydrogenase. Also, there is the potential to form a sulphate conjugate by attachment at the same alcohol group by sulphatransferases. PEG metabolism appears to be similar in animals and human and the metabolism is dependant on molecular weight with high molecular weight PEGs (>5kDa – typical of those used to PEGylate proteins) showing little or no metabolism.
Is metabolism likely to be a major route of clearance of PEG used to PEGylate proteins?

There are no data presented on the metabolism of the PEG associated with PEGylated proteins. The data presented in the previous paragraph indicate that for low molecular weight PEGs clearance by metabolism is a minor pathway. These data also demonstrate that clearance via metabolism of the PEGs which are typically used to alter the pharmacokinetics of biological products (molecular weight of 5kDa or greater (Molineux, 2003; Veronese and Pasut, 2005)) is likely to be insignificant. This is because the metabolism of PEG is molecular weight dependent with high molecular weight PEGs showing less metabolism. The primary sites of metabolic attack on PEG are frequently chemically modified, rendering metabolism unlikely (Molineux, 2003).

**Excretion of unchanged polyethylene glycol in the urine and bile: the major route of PEG clearance?**

Studies have demonstrated that PEG is eliminated in both the bile and urine in mammals, a summary of these data can be found in Table 1.

**Urinary excretion:**

Urinary excretion of PEG occurs through passive glomerular filtration and is therefore dependent on molecular weight. Studies in the mouse have shown the molecular weight dependence of the urinary elimination, with markedly reduced urinary clearance observed as the molecular weight exceeds 20kDa (Yamaoka et al., 1994). Human data also shows urinary clearance as the major excretory pathway for PEGs up to 4,000 molecular weight. Human excretion balance studies have shown that 86%
and 96% of PEG1000 and 6000 were excreted in the urine 12 hours after intravenous administration (Schaffer and Critchfield, 1947; Schaffer et al., 1950). In rats urinary recovery of PEG1000 is essentially 100% (Friman and Svanvik, 1997). In the dog PEG’s with molecular weights in the range 400-4000 are cleared from plasma at a rate identical to glomerular filtration rate (Schaffer and Critchfield, 1947), which suggests a passive filtration process.

**Biliary excretion:**

PEG also undergoes biliary excretion and this process is molecular weight-dependent with hepatic clearance reaching a minimum at about 50kDa (mouse). Lower and higher molecular weight PEGs show increased hepatobiliary clearance (Yamaoka et al., 1994). The impact of molecular weight on the hepatic and renal clearance of PEG in the mouse is shown in Figure 2. *In vitro* studies have demonstrated that the uptake of PEG by Kupffer cells increases when molecular weights increase beyond 50,000. These observations may explain the increase in hepatic clearance seen when PEG molecular weight increases over 50kDa (Yamaoka et al., 1994). Excretion studies in the bile duct cannulated dog following intravenous administration of PEG400 indicated that no PEG400 was eliminated by this route (Schaffer et al., 1950).

High biliary clearance of PEG900 in human has been reported in patients with T tube choecystectomy (Friman et al., 1995). The concentration of PEG in the bile in these studies was 31 fold higher than those seen in plasma. A similar high bile / plasma ratio has been seen in both pigs and cats with molecular weights ranging from 450-4000 (Friman et al., 1988; Friman et al., 1990). For PEG900 the biliary clearance may be a passive process. However, other authors have suggested an active component in
the biliary elimination of PEG900 involving vesicular transport via the lysozome (Roma et al., 1991). None of these studies report the urinary excretion component of PEG clearance. The data presented above indicate that for PEG urinary clearance is likely to be the major clearance mechanism (Friman and Svanvik, 1997). In the cat the maximum biliary clearance (20-25ml/h/kg) was seen with PEG900 (PEG450 had a biliary clearance of 5-10ml/h/kg, PEG2500 and 4000 was <5ml/h/kg (Friman et al., 1990)).

Excretion balance studies with PEG4000 administered intravenously to rats demonstrated that whilst urinary excretion was the major route of clearance (61% of the dose), 20% of the dose administered was present in the faeces (Carpenter et al., 1971). These data indicate for PEGs of molecular weights in the region of 4000 biliary excretion may represent a significant, but not major, clearance mechanism.

The data presented above indicate that both the liver and kidney can play a role in the excretion of PEG. However, from these studies it is clear that for molecular weights up to and including PEG190,000 urinary excretion is likely to be the major route of elimination with hepato-biliary clearance representing a minor pathway.

**Likely clearance mechanisms for PEG associated with PEGylated proteins.**

There are no data presented on the excretion of the PEG associated with PEGylated protein conjugates. Metabolic clearance of high molecular weight PEGs used for PEGylation is likely to be insignificant. The data presented above also suggest that urinary excretion of unchanged material will be the major route of clearance of any
PEG released by degradation of the conjugate. Biliary excretion of unchanged material would be expected to be a minor route.

Protein conjugates that are extensively metabolised in the hepatocyte may be an exception to this as biliary clearance may prove more a convenient clearance mechanism due to the proximity to the bile duct canalicula in the hepatocyte.

Based on the information summarised above clearance of PEG by both the biliary, urinary and metabolic routes is preceded in humans for non-conjugated PEGs and therefore is unlikely to be of any extra toxicological consequence.

**PEG exposure in humans: Pegylated proteins vs. exposures by other products.**

**Products that contain PEG.**

PEG is widely used as an excipient in medicines that are administered by the intravenous, oral, rectal and topical routes. PEG is also found in non-pharmaceutical products meaning that humans are routinely exposed to PEG. PEG containing non-pharmaceutical products include: toothpaste, shampoo, moisturisers, colorants, foods, drinks, deodorants. Exposure to PEG via these products will be by the oral and topical routes.

The absorption of PEG by the oral route is molecular weight dependent. Urinary recovery data for PEG400 indicate that 50-60% of PEG with this molecular weight is absorbed from the intestine (Schaffer et al., 1950). For PEG1000 oral absorption in humans is approximately 10%. The oral absorption of PEG6000 is very low, as no
PEG could be found in urine following oral administration. Therefore, the oral exposure to PEG is dependent on the PEG molecular weight used in a product and its dose, systemic exposure following oral administration can vary from high to very low depending upon the source of the PEG.

Topical absorption of PEG will also occur and again will show a similar molecular weight dependency as that seen orally. Absorption by this route is likely to be poor (Tsai et al., 2003). However, in burn patients administered PEG-containing ointments PEG exposure can reach significant (70µM) levels (PEG300, 1000 and 4000) (Herold et al., 1989).

A review of the toxicology information has suggested an acceptable daily intake of PEG for human. This estimate is up to 10mg/kg or 0.7g / 70kg human / day (Frujtier-Polloth, 2005). For low molecular weight PEGs this acceptable dose could in theory give rise to a systemic (absorbed) dose of approximately 400mg/day.

Exposure to PEG from intravenous medicines, comparison to those seen for PEGylated proteins.

To contextualise the potential risk associated with the administration of PEG conjugated to a protein (or other molecule) the dose of PEG received as an excipient during administration of intravenous drugs was compared with those obtained when administering a PEGylated protein at its therapeutic dose.
The data obtained in this evaluation are summarised in Table 2 and have been expressed in terms of the mass administered in order to provide an understanding of the ethylene glycol units exposure. The body burden has also been expressed as a molar amount as this represents the number of PEG molecules. These two exposure estimates allow a realistic comparison of PEG burden in human.

The data collated for this analysis demonstrate that the amounts of PEG a human is exposed to from a PEGylated biological product is significantly less than the acceptable daily intake. Even for the highest dose PEGylated protein, Somavert, the exposure of PEG in humans is approximately 25 fold lower than the estimate of acceptable human dose, based on mass. Comparison of the exposure (based on mass) of the PEGylated protein to those seen when PEG is used as an intravenous excipient, again indicates that the exposure is in all cases equal to or lower than intravenous PEG exposure. If exposure is considered on a molar basis, exposure from PEGylated biologics is at least 10 fold lower than that seen for the lowest PEG intravenous excipient dose. It should also be noted that using lorazepam for this lower end comparison is misleading as this estimate is based on a single dose and lorazepam can be administered on multiple occasions (see below). Finally, exposure to PEG from PEGylated biological products lies in a similar range to the potential exposure seen in humans for PEG impurities detailed in the blood product Venoglobin S™ (both in terms of mass and molar amounts).

The data presented above demonstrate that the PEG exposure resulting from PEGylated biologicals are comparable or lower than the intravenous exposure of PEG as an excipient. In addition to this evaluation evidence of adverse events in human
associated with PEG where sought, these data are summarised in Table 2. There have been three literature reports of PEG-related adverse events in human, these incidences are summarised below.

Intravenous nitrofurantoin (McCabe et al., 1959a) has been shown to cause acute renal tubular necrosis, oliguria and azotemia in 6/32 patients dosed for 3-5 day with a cumulative PEG dose of 121-220g (PEG300 – 400,000 to 750,000µmole). Topical nitrofurazone has shown a similar toxicity (Hunt et al., 1982). The topical cream consist of 63% PEG300 / 5% PEG1000 / 32% PEG 4000 and estimates of the concentrations in plasma associate with toxicity are in the region of 30-70mM (Herold et al., 1989). One patient who received multiple administrations of lorazepam presenting a similar toxicity (acute renal tubular necrosis) following a cumulative dose of approximately 240g of PEG400 (600,000µmole) (Laine et al., 1995).

Comparison of the exposures associated with PEG related adverse events in human with those seen from PEGylated protein indicate that exposures from PEGylated proteins are at least 600 fold lower. The major human adverse event related to high PEG exposure is renal toxicity. Renal toxicity has also been observed in the monkey at high dose indicating that similar toxicities occur across species (Fruijtier-Polloth, 2005).

Overall, the exposures of PEG from PEGylated biologicals lie below the estimate of the acceptable daily PEG dose, are no higher (and often substantially lower) than the exposures of PEG seen when PEG is used as an excipient in human and are substantially lower (>600 fold) than the exposure of PEG that is associated with
toxicity (acute renal tubular necrosis) in human. These data indicate that given the low doses used for PEGylated biological products and the PEG toxicological profile of very low concern it is unlikely that adverse events will occur in human due specifically to the PEG used to PEGylate a biological product.

**Feasibility of metabolism studies with PEGylated materials.**

Studying the metabolism of PEGylated proteins represents a significant challenge, especially *in vivo*. For small organic molecules radio-labelling is typically used to provide a selective method to identify the fate of a compound when administered to animals and human. Labelling the PEG associated with a biological molecule in order to elucidate the routes of elimination is not a viable option. Several methods exist to label PEG, these include tritium exchange, labelling the PEG with the addition of a $^{14}$C ethylene oxide or 1-Bromo-3-hydroxy-propan-2-one to the hydroxyl terminus of the PEG (Shemilt et al., 2004), iodination (Yamaoka et al., 1994) or using $\gamma$ irradiation (Leung et al., 2000). Whilst tritium exchange is a simple of radiolabelling PEG this method is none specific and the tritium may be lost through exchange with water. $\gamma$ irradiation is a destructive process that leads to a lower molecular weight species than the original PEG. The most frequent method for radiolabelling PEG requires modification of the terminal hydroxyl group. Depending on the modification of the PEG a range of radiolabels can be incorporated on to the PEG molecule, these include $^{125}$I, $^{14}$C, $^{18}$F and $^{111}$In (Yamaoka et al., 1994; Wen et al., 2003; Chen et al., 2004; Shemilt et al., 2004). These methods are generally not applicable to PEGylated proteins as radiolabelling is performed at the terminal hydroxy groups of the molecules and these groups are frequently either methylated or used to link to the protein or linker. These methods also result in the modification of the PEG structure,
limiting their usefulness for this type of investigation. This is particularly the case as the PEG hydroxyl group represents both the site of radiolabelling and the site of metabolism of the unconjugated PEGs. The addition of an extra $^{14}$C-labelled ethylene unit to the terminus of the PEG chain seems the most attractive method of labelling for PEGs. However, this frequently increases the poly dispersity of the PEG and as this is a modification at the terminus it is possible that this radiolabel could be lost leaving unlabelled PEG metabolites (Shemilt et al., 2004). Overall, unlike a small molecule, there is no readily accessible method of radiolabelling the PEG used to PEGylate proteins. Whilst radiolabelling is possible any chosen method has sufficient issues to mean that any results obtained may be difficult to interpret.

Using a specific assay to detect PEG and its potential metabolites could be considered. In order to address the metabolism question such an assay would have to have a broad cross reactivity with PEG and the acid and sulphate metabolites, ensuring all metabolites are detectable. However, humans and animals are commonly exposed to PEG via a variety of sources which means that it is likely that human plasma, urine, etc., contain a range of PEGs and PEG metabolites. When the trace doses of most PEGylated biological products are combined with the ubiquitous ‘contamination’ likely to be seen in animals and human it is unlikely that this approach to metabolite identification would be successful.

Finally identifying metabolites of PEG will also be difficult as the PEGs used have poly-dispersed molecular weight (Veronese and Pasut, 2005). Subtle changes in mass due to metabolism will be undetectable by mass spectrometry due to the range of molecular weight present in the PEG. Also work has show that can suppress
ionisation in a mass spectrometer and this may increase the uncertainty surrounding metabolite identification and quantification (Weaver and Riley, 2006).

These data indicate that the technical challenges facing an experimenter will make any experimentation in to the metabolism of PEG of little value.

**Conclusions:**

As for any other therapeutic protein being developed for administration to humans a full toxicology package in relevant species will be required for a PEGylated protein prior to commercialisation. This toxicology examination will provide a satisfactory evaluation of the toxicology associated with this molecule.

PEG has a toxicological profile of very low concern and is well tolerated at high doses following chronic and acute administration. The PEG associated with a biological molecule itself should provide no extra concern as the toxicity vs. exposure relationship in animals and human have been thoroughly investigated and metabolism / excretion is well understood. Based on the comparisons of PEG exposure from PEGylated biological products and the exposures of PEG associated with toxicity the therapeutic index is large ($\geq$600-fold). The metabolism of PEG is limited to metabolic modification of the hydroxyl group and the data available suggests that the metabolites seen in human are seen in animals. Also for PEGs typically used on biologicals, metabolism will not play a major role in PEG elimination. In light of these data PEG metabolites do not represent a significant issue, especially when combined with the low overall exposure to PEG discussed above.
Studying the metabolism of PEGylated biologics will represent a significant challenge: Firstly, radiolabelling of PEG associated with a biological molecule is not a viable option. Secondly, the doses of these PEGylated biologicals are usually very low and thirdly PEG is present in a range of products that humans are routinely exposed to. The detection of trace exposures of PEG metabolites produced from PEGylated biologicals will be impossible against the background of PEG and its metabolites present due to routine exposure. Moreover, since the products of metabolism are the same regardless of the route of administration, metabolism represents a minor route of clearance and data demonstrate that PEG exposures considerably higher than those possible from PEGylated biologicals are required for toxicity any additional experiments seem unjustified and of very limited value.

The data presented in the paper indicate that assuming toxicological evaluation of a biological molecule of interest is completed in an appropriate species and satisfactory therapeutic windows are achieved these data indicate that the PEG associated with a protein or other biological molecule do not represent a significant additional unquantified risk to humans, due to:

1. The low exposures involved
2. The low toxicity profile of PEG
3. The similarity of the metabolites that are formed in all species.

Further studies to elucidate the metabolism of the PEG associated with a biological molecule in humans will not provide any more information to place into context the safety of PEG and such studies may not even be possible.
References


McCabe W, Jackson G and Hans G (1959a) Treatment of chronic pyelonephritis. 
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McCabe W, Jackson G and HG G (1959b) Treatment of chronic Pyelonephritis II. 
Archives of Internal Medicine 104:710-719.


Figure 1: Structure of polyethylene glycol (PEG)

Figure 2: The effects of the molecular weight of PEG on urinary and hepatic clearance. (Yamaoka et al., 1994).
Table 1: Summary of PEGs metabolism and excretion data (for more detail see text).

<table>
<thead>
<tr>
<th>PEG MW</th>
<th>Urinary</th>
<th>Hepatic</th>
<th>Metabolism</th>
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<tr>
<td>&lt;1000</td>
<td>PEG1000: 86% excreted in the urine in man (1). In rat urinary recovery of PEG1000 essentially complete (11). Urinary clearance predominates (&gt;90% of the total clearance) in mouse (6).</td>
<td>PEG400: not eliminated in the bile in dogs (1). PEG900: Evidence that PEG is excreted in the bile in rats (2). PEG900: concentrations in human bile 31x higher than those in plasma, similar findings in pigs and cats (8). Hepatic clearance a minor (&lt;10%) route in mouse (6).</td>
<td>PEG400: up to 25% metabolised in human (1). PEG900: 24% of biliary excreted material PEG metabolites in cat (2). PEG1000: up to 15% metabolised in human (1).</td>
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<tr>
<td>&gt;1000</td>
<td>PEG4000: 61% of the dose eliminated in the urine of rats (9). PEG6000: 96% excreted in urine in man (1). Urinary clearance predominates (&gt;90% of the total clearance) in mouse (6).</td>
<td>PEG4000: 20% of the dose eliminated in the feces of rats (9). Hepatic clearance a minor (&lt;10%) route in mouse (6).</td>
<td>PEG6000: up to 4% metabolised in human (1).</td>
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<td>&gt;6000</td>
<td>Urinary clearance predominates (&gt;90% of the total clearance) in mouse (6).</td>
<td>Hepatic clearance a minor (&lt;10%) route in mouse (6).</td>
<td>Conclusion: Acid and hydroxyl acid metabolites seen in plasma and urine of animals and man (3). Sulphation observed in animals (4). Alcohol dehydrogenase and P450 have been reported to be responsible for the metabolism of PEG (5). Metabolism represents a minor route of elimination for PEG.</td>
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Comments and Conclusion

In dog PEG400-4000 cleared at a rate equivalent to GFR (10). **Conclusion:** Urinary clearance predominates for PEG’s up to 190,000 molecular weight. High molecular weight PEGs can be excreted into the urine.

(1) (Schaffer and Critchfield, 1947; Schaffer et al., 1950)
(2) (Friman et al., 1993)
(3) (Hunt et al., 1982; Friman et al., 1990; Friman et al., 1993)
(4) (Roy et al., 1987; Roy et al., 1988)
(5) (Herold et al., 1989; Veronese and Pasut, 2005)
(6) (Yamaoka et al., 1994)
(7) (Friman et al., 1988; Friman et al., 1990; Friman et al., 1995)
(8) (Carpenter et al., 1971)
(9) (WHO Food Additives Series Number 14)
(10) (Friman and Svanvik, 1997)
Table 2: Comparison of the estimated PEG exposures in human resulting from PEGylated protein or as an excipient in intravenous medicines.

<table>
<thead>
<tr>
<th>Compound</th>
<th>PEG details</th>
<th>Estimated body burden</th>
<th>Comment</th>
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<tr>
<td></td>
<td></td>
<td>mg / week</td>
<td>μmole / week</td>
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<tr>
<td>Doses of PEG associated with acute renal failure in patients</td>
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<td>Nitrofurantoin</td>
<td>PEG300</td>
<td>121,000-220,000</td>
<td>400,000 – 750,000</td>
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<td>Lorazepam</td>
<td>PEG400</td>
<td>240,000</td>
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<td>Intravenous drugs containing PEG as an excipient</td>
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<td>Busulfex™ (Busulfan)</td>
<td>PEG400</td>
<td>110,000</td>
<td>275,000</td>
</tr>
<tr>
<td>Vepesid™ (Etoposide)</td>
<td>PEG300</td>
<td>15,000</td>
<td>51,000</td>
</tr>
<tr>
<td>Dipyridamole</td>
<td>PEG600</td>
<td>400</td>
<td>670</td>
</tr>
<tr>
<td>Ativan™ (Lorazepam)</td>
<td>PEG400</td>
<td>200</td>
<td>500</td>
</tr>
<tr>
<td>Human globulin (Venoglobin-S™)</td>
<td>Not stated, assumed PEG400.</td>
<td>≤28-280</td>
<td>≤70-700</td>
</tr>
<tr>
<td>Aralast™</td>
<td>Not stated, assumed PEG400.</td>
<td>≤0.6</td>
<td>≤1.5</td>
</tr>
<tr>
<td>PEGylated biological products</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Somavert™</td>
<td>Multiple (4-5) 5kDa PEG</td>
<td>200</td>
<td>40</td>
</tr>
<tr>
<td>Neulasta™</td>
<td>Single 20kDa monomethoxy PEG</td>
<td>6</td>
<td>0.3</td>
</tr>
<tr>
<td>Pegasys™</td>
<td>40kDa branched bis methoxy PEG</td>
<td>0.18</td>
<td>0.005</td>
</tr>
<tr>
<td>PEG-Intron™</td>
<td>Single 12 kDa monomethoxy PEG</td>
<td>0.11</td>
<td>0.006</td>
</tr>
</tbody>
</table>

Other intravenous products and PEGylated proteins are available but insufficient data could be found to allow estimation of exposure. These included Adagen™, Oncaspar™, and Torsemide™.

Assumptions
Data estimated from products package insert or label (see WWW.FDA.GOV)
Highest dose presented in the insert was used in the estimate.
Specific gravity of PEG is 1.1 g/ml.
\[
\begin{align*}
&\text{HO-} - \underbrace{\text{O-}}_{n}\text{OH} \\
&\text{HO-} - \underbrace{\text{O-}}_{n}\text{O-} - \text{OMe}
\end{align*}
\]