CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

204630Orig1s000

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW(S)

CLINICAL PHARMACOLOGY REVIEW

NDA Number:	204630
Submission Type; Code:	Complete Response Resubmission
Applicant Name:	Provepharm SAS
Submission Dates (SDN):	10/09/2015 (48), 10/29/2015 (50), 11/13/2015 (53), 02/10/2016 (60), and 02/29/2016 (62)
Brand Name:	(b) (4)
Generic Name	Methylene Blue
Dosage Form:	Sterile Solution for Intravenous Administration
Dosage Strengths:	0.5% (5 mg/mL) Methylene Blue Solution in a 10 mL Ampule
Proposed Indication:	The Treatment of Acquired Methemoglobinemia (b) (4)
OCP Division:	DCP V
Primary Reviewer:	Salaheldin S. Hamed, Ph.D.
Team Leader:	Bahru Habtemariam, Pharm D.

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1 EXECUTIVE SUMMARY

[redacted] Injection, 0.5% (methylene blue) is an oxidation-reduction agent for the treatment of acquired methemoglobinemia (metHb). The proposed dose is 1 mg/kg given as a 5-minute intravenous infusion.

The applicant submitted efficacy data from a retrospective study in patients with acquired metHb (N=12) who were treated with [redacted] doses of 1 or 2 mg/kg in 9 hospitals in France and the UK. The applicant also provided extensive literature reports and case study reports for the use of methylene blue (MB) in the treatment of acquired metHb. Efficacy results are summarized below:

- Retrospective study in 12 subjects (foreign sourced [redacted]
  - metHb reduced from 40.7±18% to less than 3% in 5 patients
  - metHb eventually resolved in all patients.
- Literature case reports (Dr. Przepiorka Clinical Review, DARRTS date 8/19/2014):
  - 38 out of 42 cases met primary endpoint (50% reduction of MetHb at one hour post infusion)
  - 1 mg/kg: response rate 86% (60-96%) (n=14)
  - 2 mg/kg: response rate 91% (62-98%) (n=11)

The limited retrospective and literature data indicate there is no increased efficacy at doses greater than 1 mg/kg. Therefore, the review team is recommending a dose of 1 mg/kg.

In terms of safety, case reports indicate adverse events and fatal toxicities occurred when MB was given to patients taking anti-depressants, including selective serotonin reuptake inhibitor (SSRI). These anti-depressants are metabolized by CYP450 enzymes.

To establish a PK bridge to the efficacy/safety data reported in the literature, the applicant conducted a single dose parallel BE study comparing the PK of [redacted] to the MB 1% USP product widely used in the clinical studies reported in the literature. The BE study was conducted at a nominal dose of 2 mg/kg dose.

The BE study results indicate that the dose normalized AUC of MB falls within the acceptance bounds (**Table 1**) whereas the dose normalized C_{max} 90% CI bounds fall outside the acceptance criteria. The difference in C_{max} between the two products was due to the presence of reduced amount of active ingredient in the reference product. Thus, from a clinical pharmacology perspective, the study established a PK bridge between [redacted] and MB 1% USP.

Table 1: Summary of Methylene Blue BE Study Results

Study	Mehtylene Blue	Geometric Mean Ratio (90% CI)
Whole Blood	AUC_{inf}/Dose	104 (96,113)
	C_{MAX}/Dose	115 (96, 137)
Plasma	AUC_{inf}/Dose	103 (96, 112)
	C_{MAX}/Dose	87 (82,96)

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ADME information in the submitted literature reports suggests that MB undergoes hepatic and renal clearance; however, the contribution of renal and hepatic routes in the elimination of MB has not been determined. As a result, dosing recommendations in patients with renal or hepatic impairment cannot be made. Since higher doses (>2 mg/kg) of MB have been associated with hemolytic anemia and other severe adverse events, the influence of renal and hepatic impairment needs to be assessed in subjects with varying degrees of renal and hepatic impairment.

In vitro incubation with human hepatocytes resulted in ~33% metabolism of methylene blue. Further in vitro studies suggested that UGT1A9, UGT1A4, CYP1A2, CYP2D6, and CYP2C19 had the capacity to metabolize MB.

In vitro studies with CYP450 enzymes indicate that MB is a potent inhibitor of a number of CYP450 enzymes (CYP1A2, 2C8, 2C19, 3A4, 2B6, 2D6, and 2C9). The R values, which are calculated as the ratio of the C_{max} relative to the k_i in vitro, ranged from 4 to 60, indicating the need for in vivo DDI studies. In addition, the published reports of fatal drug-drug interactions with the anti-depressant drugs further support the need for in vivo drug-drug interaction (DDI) evaluations.

1.1 Recommendations

This NDA is acceptable from a clinical pharmacology perspective provided that the applicant and the agency come to an agreement regarding the labeling language and the identified clinical studies under the post-marketing requirements (PMRs).

Decision	Acceptable to OCP?			Comment
Overall	Yes ☒	No ☐	NA ☐	Approvable for the treatment of methemoglobinemia based on the retrospective efficacy study and adequate scientific bridging to efficacy reports in the medical literature.
Evidence of Effectiveness	Yes ☒	No ☐	NA ☐	1 retrosepctive efficacy study and reports of efficacy from the medical literature
Proposed dose for general population	Yes ☒	No ☐	NA ☐	1 mg/kg initial dose and repeat dose of 1 mg/kg if symptoms are not resolved, within one hour of the initial dose.
Proposed dose selection for others	Yes ☐	No ☐	NA ☒	Dose adjustments in patients with renal or hepatic impairment are to be determined based on PMR trials.
Pivotal BE	Yes ☒	No ☐	NA ☐	A pivotal BE study was conducted, the PK exposure was comparable between (b) (4) and MB 1% USP.
Labeling	Yes ☐	No ☒	NA ☐	Revised labeling is proposed

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1.2 Post Marketing Requirements

(b) (4)

(b) (4)

(b) (4)

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DCP V: Reviewer – **S. Hamed**; TL – **B. Habtemariam**; DDD – **B. Booth**;
DD – **A. Rahman**

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Summary of Important Clinical Pharmacology and Biopharmaceutics Findings

[redacted] is an oxidation-reduction agent that reduces the oxidized iron (Fe3+) in methemoglobin to the reduced form (Fe2+) in normal hemoglobin. The applicant is seeking the approval of [redacted] for the treatment of acquired methemoglobinemia.

NDA 204630 was submitted as a 505(b)(2) application; initially, the applicant relied on the published literature to demonstrate safety and efficacy of MB in the treatment of metHb and data from a retrospective study in 12 metHb patients treated with [redacted] However, there was no sufficient relative bioavailability (BA) information to bridge the PK of MB 1% USP used in the majority of the reported trials and [redacted] In addition, the applicant did not provide any clinical pharmacology studies for the proposed product. A Complete Response was issued for lack of information regarding the PK of [redacted] and the ADME properties of MB in general (see Clinical Pharmacology Review by Dr. Joseph Grillo, DARRTS date 09/04/2014).

In the present submission, the applicant provided a relative BA/BE study comparing the PK of [redacted] to MB 1% USP after a nominal dose of 2 mg/kg, a QT prolongation trial, [redacted] and in vitro metabolism and DDI studies.

The applicant conducted a single dose, parallel BE study comparing the PK of MB 1% USP and [redacted] The concentrations of MB and Azure B, the major metabolite, were measured in whole blood and plasma. The dose adjusted AUC in whole blood was within the bioequivalence bounds; however, the dose adjusted [redacted] C_{max} was higher than that of the USP solution (**Table 1**).

It was noted during the conduct of the study that the reference formulation was administered at ~ 1.6 mg/kg dose instead of the nominal 2 mg/kg dose. Methylene blue exhibits concentration-dependent partitioning into red blood cells, with higher partitioning at higher concentrations. The concentration-dependent partitioning is likely to be the reason for the observed differences in the dose adjusted C_{max} between the two products.

The ADME properties of MB are not very well characterized in humans. Literature studies report variable PK characteristics of MB, mainly due to varying sampling times and assay selectivity issues. Recent reports that employ more selective bioanalytical methods suggest 40% of the MB dose is excreted by renal elimination as parent drug and the balance of the dose undergoes hepatic metabolism.

In vitro metabolism data demonstrate that MB is metabolized in human hepatocytes (~33% of the initial MB amount). In vitro incubation of methylene blue with human liver microsomes suggested that UGT enzymes played a more prominent role in metabolism compared to CYP450 enzymes. To characterize the role of individual metabolizing enzymes, MB was incubated with human liver microsomes expressing individual recombinant CYP450 or UGT enzymes in vitro. It was found that CYP1A2, CYP2D6, CYP2C19, UGT 1A9, and UGT 1A4 had the capacity to metabolize MB. The relative contribution of the individual enzymes or elimination pathways is not thoroughly

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characterized. However, the multiplicity of the elimination pathways and metabolizing enzymes preclude the need for in vivo DDI studies of MB as a victim drug.

In vitro studies show that MB is a potent inhibitor of CYP450 1A2, 2C8, 2C19, 3A4, 2B6, 2D6, 2C9 with R values of 59, 43, 32, 21, 11, 9, and 4, respectively. Even with the acute dosing regimen of MB, in vivo DDI study of MB as a perpetrator drug are warranted due to the relatively long elimination half-life (~24 hours).

Based on published safety communications by the FDA, drug interactions of MB with selective serotonin reuptake inhibitors (SSRI) and other anti-depressants (bupropion, buspirone, venlafaxine, etc.) have been reported to be fatal. The fatality of these interactions has been attributed to the pharmacodynamic interaction of MB as a monoamine oxidase inhibitor with SSRI. Because it has not been formally investigated, the contribution of pharmacokinetic interactions of MB as an inhibitor of many CYP450 enzymes involved in the metabolism of SSRI and other anti-depressants cannot be ruled out. To mitigate the risk for drug-drug interactions, an in vivo study of MB as a perpetrator drug is warranted.

The applicant did not submit data regarding dosing in special populations. (b) (4) The pediatric dosing will be based on efficacy studies reported in the literature, which is under the purview of the clinical review team.

(b) (4)

(b) (4) In addition, there is no conclusive evidence that renal clearance is the primary route of elimination. Similarly, the contribution of hepatic clearance to the overall elimination of MB has not been determined. Since higher doses (>2 mg/kg) of MB have been associated with hemolytic anemia and other severe adverse events, in vivo studies are warranted to support dosing recommendations in patients with hepatic or renal impairment

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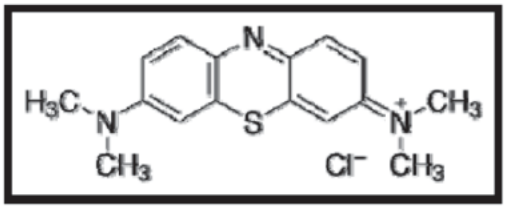
2 QUESTION BASED REVIEW

2.1 General Attributes of the Drug

Information provided in this section are taken directly from Dr. Joseph Grillo's Clinical Pharmacology review in DARRTS (09/04/2014)

2.1.1 What are the highlights of the chemistry and physical-chemical properties of the drug substance and the formulation of the drug product?

Methylene blue is a water-soluble thiazine dye. The molecular formula of MB is $C_{16}H_{18}ClN_3S$. Its structural formula is:



[redacted] is a sterile solution intended for intravenous administration. Each [redacted] Injection, 0.5%, 10 mL ampule contains 50 mg methylene blue and water for injection (q.s.) resulting in each 1 mL of solution containing 5 mg methylene Blue. [redacted] Injection, 0.5% is a clear dark blue solution with a pH value between 3.0 and 4.5. The osmolality is between 10 and 15 mOsm/kg.

[redacted] is not the same concentration as MB 1% USP that is currently used clinically as an unlicensed pharmaceutical. The applicant states that the manufacturing process [redacted]

2.1.2 What are the proposed mechanism of action and therapeutic indications?

The applicant is proposing that [redacted] be indicated as an antidote for the treatment of acquired methemoglobinemia ([redacted]. This indication includes both adult and pediatric patients.

The mechanism of action of MB for the proposed indication (**Figure 1**) [redacted] In turn, LMB promotes a non-enzymatic redox reaction of methemoglobin (metHb) to hemoglobin. Since MB is itself an oxidizer, at higher concentration it is thought to have a paradoxical effect and promote metHb generation.

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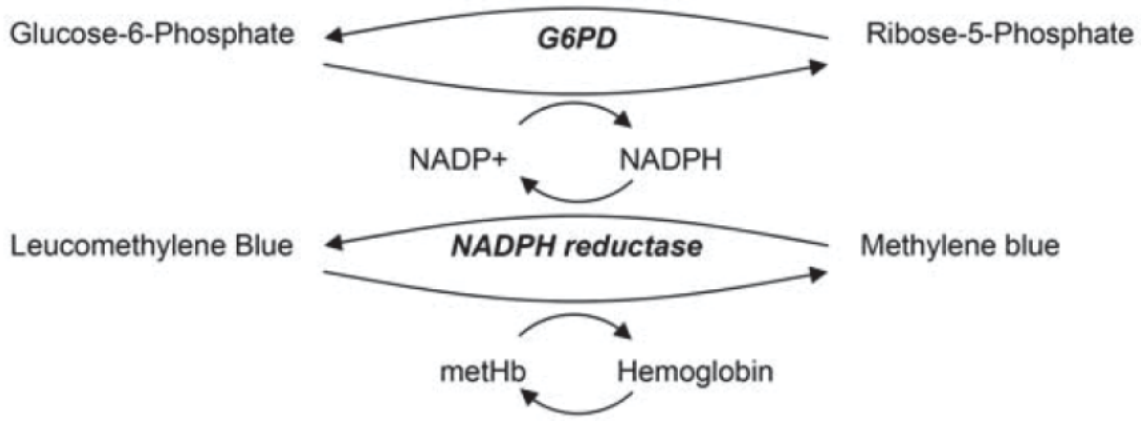

Figure 1: Reduction of methemoglobin to hemoglobin by NADPH reductase

2.1.3 What are the proposed dosages and routes of administration?

(b) (4) is administered intravenously as a slow bolus injection over a period of 5 minutes or diluted in 5% dextrose in water (D5W). The applicant proposes the dosing regimen described in **Table 2**.

Table 2: Proposed (b) (4) Dosing

(b) (4)

Source: Applicant's proposed labeling

2.2 General Clinical Pharmacology

2.2.1 What are the design features of the clinical pharmacology and the clinical studies used to support dosing or claims?

To support dosing in adults and pediatric patients, the applicant submitted extensive literature reports for the use of methylene blue in the treatment of acquired metHb. In addition, the applicant provided efficacy data from a retrospective study (Study PVP-2013001); safety data through a compassionate use program (PVP-2013002); PK data to demonstrate the bioequivalence of (b) (4) and MB 1% USP (PVP-2014001); PK and safety data of MB 1% USP (Study 1MBL12002); and QT prolongation data for (b) (4) (Study PVP-2014002). The clinical studies in support of the application are summarized in **Table 3**.

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Table 3: Clinical Studies Submitted by the Applicant

Trial (N)	Design	Population	1º Endpoint
PVP-2013001 (N=12)	- Retrospective chart review - (b) (4) (dose not prespecified)	Patients treated with (b) (4) for metHb	At least 50% reduction of MetHb at one hour after infusion
PVP-2013002 (N=27)	- Compassionate use program - (b) (4) 1-2 mg/kg hourly up to 7 mg/kg	Adults and children >3 mo old with metHb	Safety
PVP-2014001 (N=70)	Single dose, parallel BE study of (b) (4) vs. MB 1% USP (1:1 randomization) at 2 mg/kg dose	Healthy subjects	- BE of (b) (4) and MB 1% US - Safety and tolerability of (b) (4)
1MBL12002 (N = 12)	- Single dose study - MB 1% USP Product (1mg/kg)	Healthy subjects	- MB PK - Safety
PVP-2014002 (N=48)	-Crossover study of positive control/ placebo and (b) (4) on QT prolongation (b) (4) 2 mg/kg (n = 16) -Moxifloxacin 400 mg p.o (n =16) -Placebo (saline) (n=16)	Healthy subjects	- Effect of (b) (4) on QTc interval - Safety of (b) (4)

The retrospective efficacy study was conducted in nine hospitals in France and the UK. The patient demographics, treatment regimen, and outcome are summarized in **Table 4**.

Table 4: Retrospective Study (PVP-2013001) Results. Pediatric Patient Data are in Boldface.

Subject	Age (year)	Sex	Weight (kg)	First Dose (mg)	First Dose (mg/kg)	Total Dose (mg)	Total Dose (mg/kg)	No. doses	Treatment Duration (h)	MetHb at presentation	Final MetHb
1	26	F	66	120	2	420	**7**	**4**	NR	43.7	0.7
2	28	F	60	60	1	60	1	1	1	10.6	1.4
3	37	F	61	120	2	780	**13**	**3**	12.5	75	0.9

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4	**0.016**	**F**	**1.73**	**2**	**1**	**2**	**1**	**1**	**0.5**	**10.7**	**1.4**
5	52	M	100	100	1	100	1	1	0.25	32	
6	40	M	70	70	1	70	1	1	0.25	29.6	2.8
7	37	M	80	80	1	80	1	1	0.25	42	NR
8	26	M	70	70	1	70	1	1	0.25	63	NR
9	54	M	70	70	1	70	1	1	NA	43.9	NR
10	25	M	65	50	0.77	100	1.54	2	0.1667	47	0
11	**1**	**F**	**11**	**22**	**2**	**22**	**2**	**1**	**2**	**44.4**	**0.8**
12	**2**	**F**	**14.2**	**25**	**2**	**25**	**2**	**1**	**0.5**	**43.5**	**0.7**

2.2.2 What is the basis for selecting the response endpoints and how are they measured in clinical pharmacology studies?

In the retrospective trial PVP-2013001, the efficacy endpoint was 50% reduction of metHb level in the blood within one hour. In the literature reports, other endpoints were used, such as symptomatic improvement and arterial blood gases, in addition to the metHb levels.

2.2.3 Are the active moieties in plasma appropriately identified and measured to assess pharmacokinetic parameters and exposure response relationships?

The applicant submitted a validated bioanalytical study report. In the BE study, the concentration of MB as well as the major metabolite Azure B were measured in plasma and whole blood.

2.2.4 Exposure-Response

2.2.4.1 What are the characteristics of the exposure-response relationships for efficacy?

No exposure-response data were submitted in this NDA.

2.2.4.2 What are the characteristics of the exposure-response relationships for safety?

No exposure-response data for safety were submitted in this NDA.

2.2.4.3 Does this drug prolong QT/QTc Interval?

(b) (4) does not exhibit significant QTc prolongation at a single dose of 2 mg/kg IV dose. However, the effect of supra-therapeutic doses was not studied. The IRT review

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contains detailed assessment of the QT study submitted by the sponsor (Review by Moh Jee Ng, 2/10/2016).

2.2.4.4 Is the dose and dosing regimen selected by the sponsor consistent with the known E-R relationship?

There was no E-R relationship to support dosing. MB doses of 1-2 mg/kg IV are used empirically in the cases of acquired metHb. The proposed dosing is based on the safety and efficacy endpoints from the submitted retrospective study in 12 patients, pharmacokinetic studies, and reports in the medical literature.

2.2.5 What are the PK characteristics of the drug?

The PK data obtained from the clinical studies submitted by the applicant show a C_{max} of ~3000 ng/mL is reached at the end of a 5-min infusion of a 2 mg/kg dose of (b) (4) The PK profile of MB is multiphasic with a terminal elimination half-life of approximately 24 hours (**Figure 2**). Azure B, (b) (4) is the major metabolite identified for MB. Azure B AUC is < 20% when compared to that of MB. The steady state volume of distribution is 255-299 L, suggesting that MB distributes to tissues. MB exhibits concentration-dependent partitioning into red blood cells, with higher blood-to-plasma ratio at higher concentration. MB was 94-97% bound at 10 micromolar concentration in vitro. The PK parameters following a single 2 mg/kg dose are summarized in **Table 5**.

Table 5: PK Parameters of (b) (4) and Metabolite after a 2 mg/kg Dose

PARAMETER (units)	(b) (4) (%CV)	Azure B (%CV)
AUC_{0-t} (ng*hr/mL)	13977 (21.1)	2050.1 (26.3)
AUC_{0-t}/Dose (ng*hr/mL/mg)	100.27 (19.0)	
AUC_{inf} (ng*hr/mL)	15668 (23.7)	2466.4 (25.5)
AUC_{inf}/Dose (ng*hr/mL/mg)	112.4 (22.2)	
C_{max} (ng/mL)	2917 (38.6)	104.6 (24.0)
C_{max}/Dose (ng/mL/mg)	20.92 (37.2)	
$T_{1/2}$ (h)	23.49 ± 6.555	23.3 ± 6.54
CL (L/h)	9.1 ± 1.98	
V_{ss}	255.1 ± 58.18	

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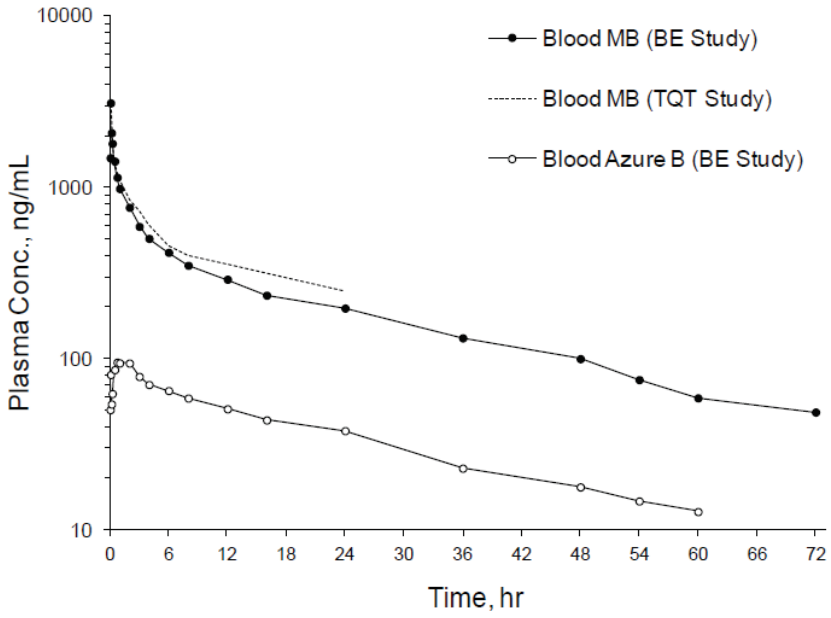

Figure 2: (b) (4) and metabolite PK profiles in whole blood. Data were collected from the BE study and the TQT study.

In addition to the PK data submitted, the applicant provided information regarding the PK of MB 1% solution from the literature. The PK data reported in these studies were highly variable, primarily due to sampling times and the matrix (blood versus plasma) in which the MB was measured. The reported half-lives in these studies were highly dependent on the terminal sampling time due to the multiphasic nature of the MB PK.

2.2.5.1 What are the single and multiple dose PK parameters?

Due to the acute dosing of MB in the treatment of acquired metHb, multiple dose PK parameters were not determined.

2.2.5.2 How does the PK of the drug and its major metabolites in healthy adults compare to that in patients?

The PK of MB and Azure B were determined in normal healthy volunteers only. However, there is no evidence to suggest that the PK is expected to be different in patients with acquired metHb.

2.2.5.3 What are the characteristics of drug absorption?

(b) (4) is administered as an IV infusion over a period of 5 minutes.

2.2.5.4 What are the characteristics of drug distribution?

The observed volume of distribution of (b) (4) is 255-299 L, suggesting that methylene blue is well-distributed into the tissue compartment. In vitro binding studies indicate that the drug is ~95% bound to plasma proteins at a concentration of 10 μM (3200 ng/mL). Methylene blue exhibits concentration-dependent partitioning into red blood cells in vitro (**Figure 3**) and in vivo (**Figure 4**). In vitro, the blood-to-plasma ratio increases to ~50-fold at 5000 ng/mL. The blood-to-plasma ratio exhibits concentration-

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dependent change in vivo in the case of MB 1% USP (blue bars) and [redacted] (red bars).

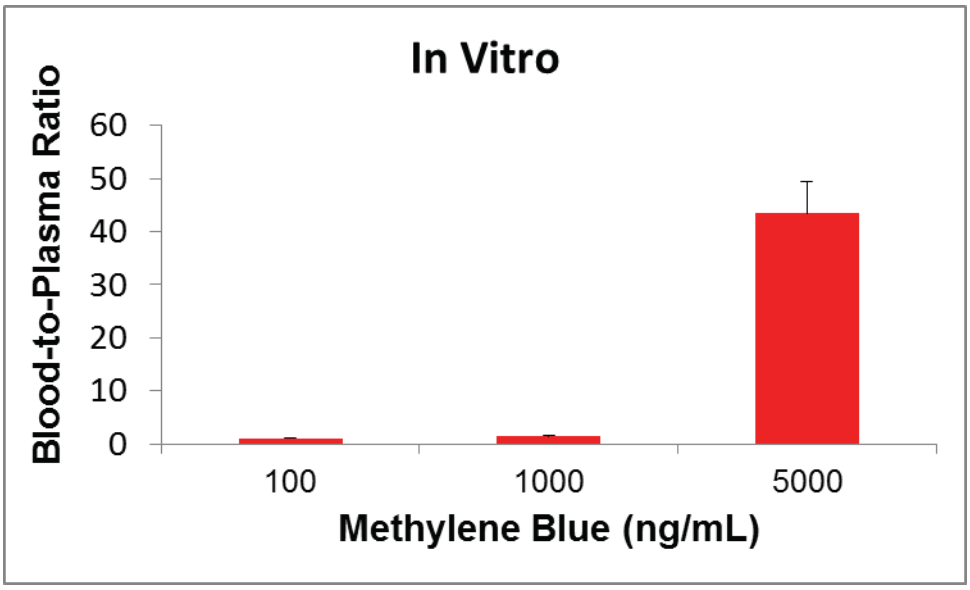

Figure 3: Blood-to-plasma Ratio of methylene blue in vitro.

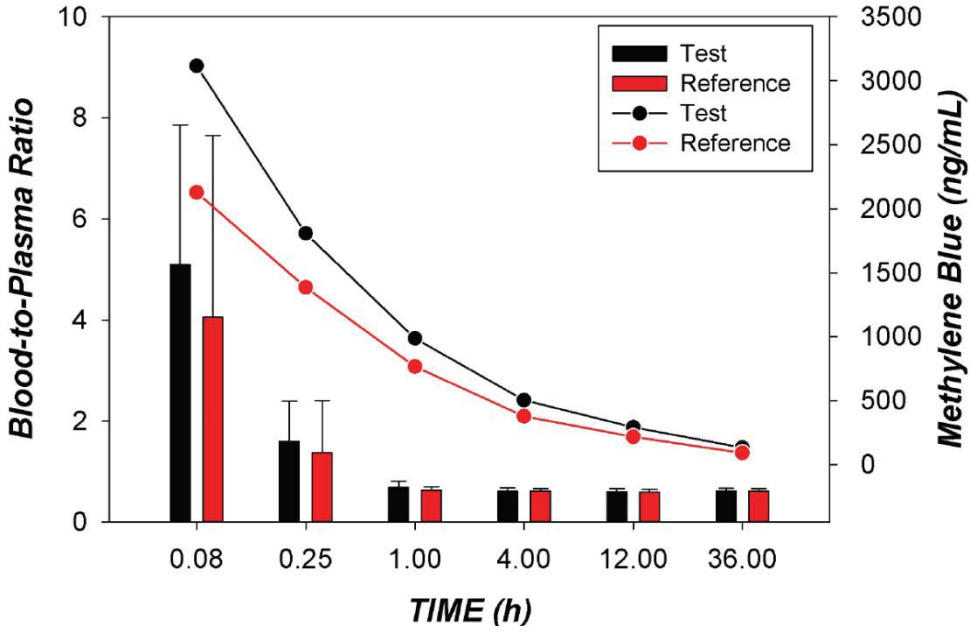

Figure 4: Blood-to-plasma ratio of [redacted] (Test) and MB 1% USP (reference) is represented with bars. Blood concentrations are represented with lines. At high concentrations, the blood-to-plasma ratio is high; subsequently, when the blood concentration is low, the blood-to-plasma ratio reaches a plateau.

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2.2.5.5 Does the mass balance study suggest renal or hepatic as the major route of elimination?

The applicant did not submit mass balance studies.

2.2.5.6 What are the characteristics of drug metabolism?

Methylene blue metabolism studies were carried out in vitro in human hepatocytes. The only metabolite identified was Azure B. In vitro studies of MB incubated with individual recombinant enzymes demonstrated that MB is extensively metabolized by CYP1A2 (99% of initial MB amount), CYP2D6 (75%), CYP2C19 (58%), UGT1A9 (91%), and UGT1A4 (44%). In human liver microsomes, approximately 30% of the initial MB amount was metabolized by CYP450 enzymes (in the presence of NADPH cofactor) and 80% was metabolized by UGT enzymes (in the presence of PDGA cofactor). In hepatocytes, however, the extent of MB metabolism was ~33%.

2.2.5.7 What are the characteristics of drug elimination?

Information published in the literature suggests that renal elimination plays a role in the clearance of MB. The total radioactivity retrieved in the urine was found to be ~ 30% at 24 hours in one study. In another study, the unchanged amount recovered in the urine was 40% at 72 hours. In vitro, MB was found to be metabolized in human hepatocytes as well. The contribution of each pathway is not well characterized.

2.2.5.8 What is the inter- and intra-subject variability of PK parameters in volunteers and patients?

The inter- and intra-subject variability of PK parameters is summarized in **Table 6**. The inter-individual variability was < 18% for all parameters and was not explained by any of the covariates investigated.

Table 6: Population PK Parameter Estimates of Methylene Blue in Healthy Adults

Parameters	Estimates	RSE (%)
CL (L/h/70kg)	9.1	4%
V1 (L/70kg)	39.4	9%
Q2 (L/h/70kg)	27.8	8%
V2 (L/70kg)	167	5%
Q3 (L/h/70kg)	147	27%
V3 (L/70kg)	39.3	9%
Between subject variability		
ω^2CL	0.046	12%
ω^2V1	0.195	14%
ω^2Q2	0.192	18%
ω^2V2	0.0846	16%
Residual error		
σ^2_{Prop}	0.0283	6%

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2.3 Intrinsic Factors

2.3.1 What intrinsic factors influence exposure and/or response, and what is the impact of any differences in exposure on efficacy or safety responses?

The applicant conducted population PK analysis using data obtained from 35 patients in the BE study who were given a 2 mg/kg dose of (b) (4) The applicant investigated the effect of age, weight, gender, ethnicity, and race as intrinsic factors. None of the investigated factors were found to affect exposure.

2.3.1.1 Pediatric Patients

(b) (4)

2.3.1.2 Race

Based on the population PK modeling, race did not affect the PK of methylene blue.

2.3.1.3 Renal Impairment

No studies were conducted in subjects with renal impairment.

2.3.1.4 Hepatic Impairment

No studies were conducted in subjects with hepatic impairment.

2.3.2 What pregnancy and lactation use information is there in the label?

The label states (b) (4) For nursing patients, the label recommends discontinuing breastfeeding up to 8 days after treatment.

2.4 Extrinsic Factors

2.4.1 What extrinsic factors influence exposure and/or response, and what is the impact of any differences in exposure on efficacy or safety responses?

There were no trials designed to evaluate the effect of extrinsic factors on the pharmacokinetics of methylene blue.

2.4.2 What are the drug-drug interactions?

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2.4.2.1 Is there an in vitro basis to suspect in vivo drug-drug interactions?

In vitro, methylene blue was found to be a potent inhibitor of a number of CYP450 enzymes based on the R value (**Table 7**). The terminal half-life of MB is relatively long (~24 hours), which suggests that MB may affect the elimination of drugs that are CYP450 enzyme substrates. The R value for each CYP450 enzyme inhibition based on the unbound fraction (Ru) is greater than the threshold of 1.02. **Figure 5** shows the time needed, based on plasma concentration, to reach an R value <1.1 for each of the enzymes that are inhibited by MB.

Table 7: In Vitro Inhibition of CYP450 Enzymes and Potential DDI Prediction

CYP	IC_{50} (ng/mL)	k_i	R	R (u)
1A2	43	21.5	58.9	2.7
2B6	262	131	10.5	2.2
2C8	60	30	42.5	1.9
2C9	826	413	4.0	1.6
2C19	81	40.5	31.7	1.6
2D6	326	163	8.6	1.3
3A4 (M)	122	61	21.4	1.2
3A4 (T)	128	64	20.4	1.1

Reference ID: 3903201

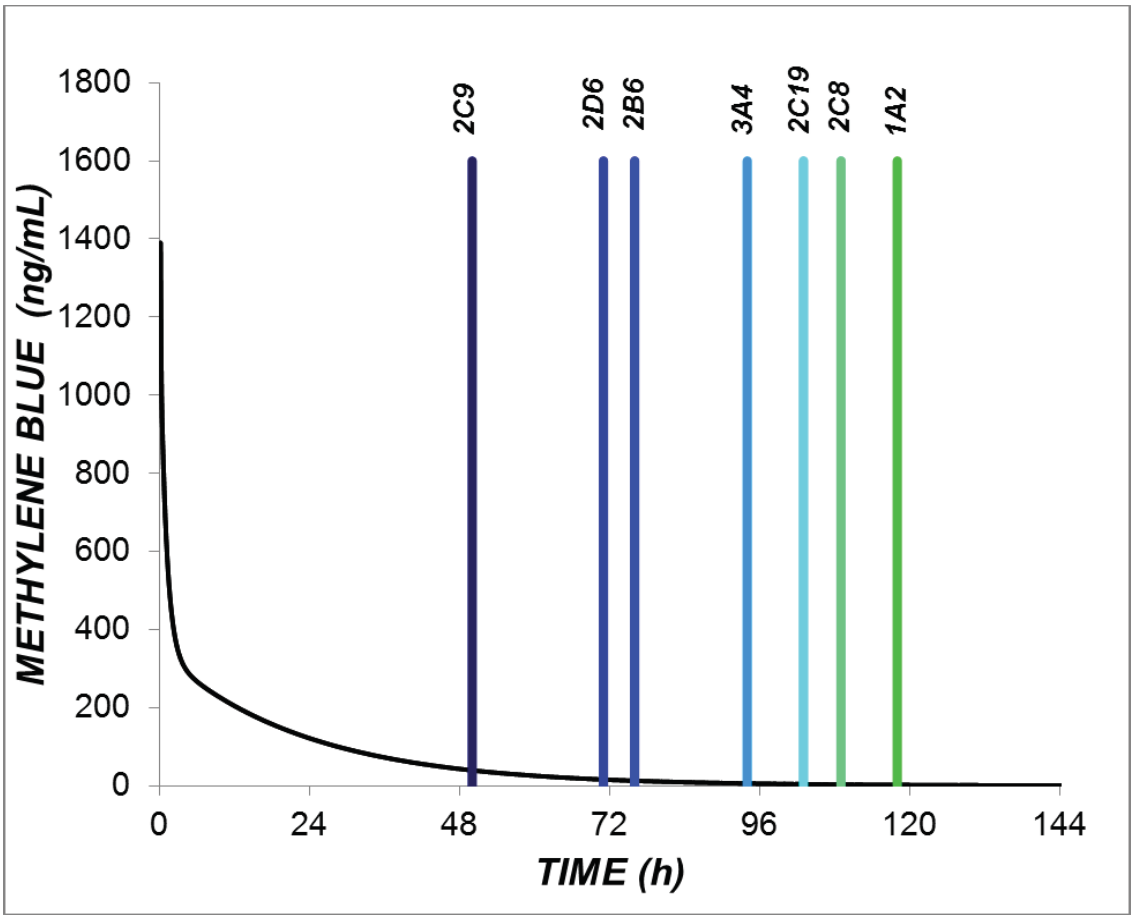

Figure 5: The time needed, based on single IV dose of 1 mg/kg MB, to reach a blood concentration of MB that would render DDI insignificant (i.e. R <1.1) for each of the enzymes inhibited by MB.

2.4.2.2 Is the drug a substrate of CYP enzymes? Is metabolism influenced by genetics?

In vitro experiments indicate that MB is metabolized in human hepatocytes and human liver microsomes (~30% of metabolism is attributed to CYP450 enzyme activity). Incubation with recombinant CYP450 enzymes resulted in 91% metabolism of MB with CYP1A2, 75% metabolism with CYP2D6, and 58% metabolism with CYP2C19.

2.4.2.3 Is the drug an inhibitor and/or an inducer of CYP enzymes?

In vitro experiments indicated that methylene blue as an inhibitor of a number of CYP450 enzymes (**Table 7**).

2.4.2.4 Is the drug an inhibitor and/or an inducer of PGP transport processes?

Bidirectional permeability studies of digoxin in Caco-2 cells indicated at MB is an inhibitor of P-gp with an IC_{50} of 64.6 μM (18732 ng/mL); the $[I]/IC_{50}$ is less than 0.1.

Reference ID: 3903201

2.4.2.5 Are there other metabolic/transporter pathways that may be important?

Incubation studies in human liver microsomes indicated that MB was 80% metabolized by UGT enzymes. Incubation with recombinant UGT enzymes resulted in 91% metabolism of MB with UGT1A9 and 44% metabolism with UGT1A4.

2.4.2.6 Are there any unresolved questions related to metabolism, active metabolites, metabolic drug interactions, or protein binding?

The contribution of renal elimination and hepatic metabolism to the overall elimination of MB has not been characterized. In addition, the plasma protein binding in vitro study was conducted at only one concentration (10 μM or ~3000 ng/mL). Non-linear binding to plasma protein may explain the concentration-dependent partitioning into blood cells.

2.5 General Biopharmaceutics

2.5.1.1 What are the safety or efficacy issues, if any, for BE studies that fail to meet the 90% CI using equivalence limits of 80-125%?

A single dose, parallel bioequivalence study was conducted to compare the PK of MB and Azure B in whole blood and plasma following the administration of 2 mg/kg dose. A total of 35 normal healthy volunteers were included in each arm of the study. Upon examination of the study conduct by this reviewer, it was found that the reference formulation (MB 1% USP) was given at an approximate dose of 1.6 mg/kg, whereas the reference was administered at the nominal dose (2 mg/kg) (**Figure 6**). The sponsor provided analytical chemistry data showing that the content of active ingredient in the MB 1% USP product is 80% of the nominal value.

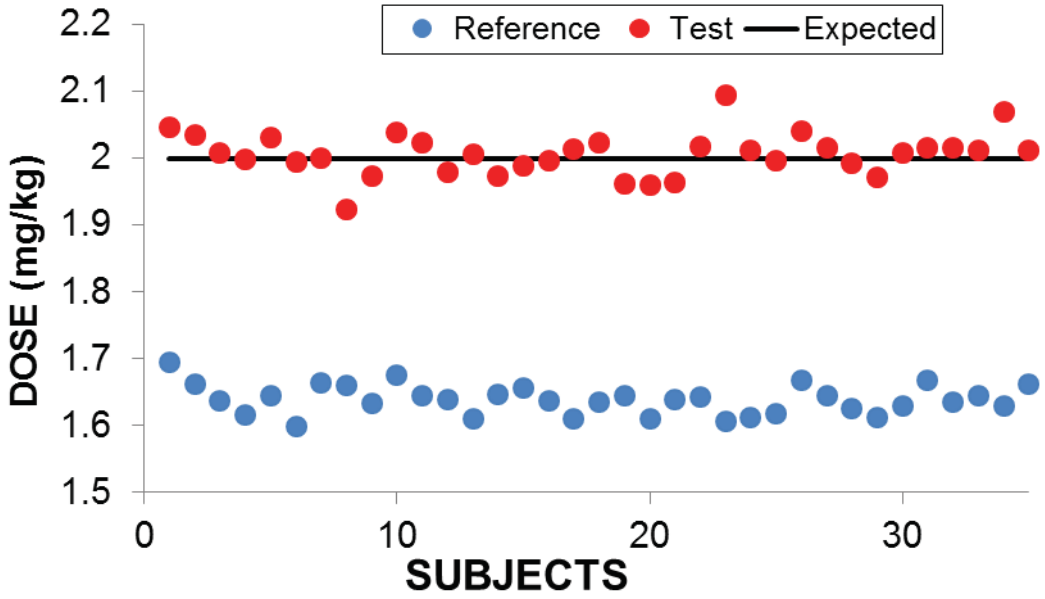

Figure 6: Doses administered to 35 subjects in each arm of the parallel BE study. The reference formulation (MB 1% USP) was administered at 1.6 mg/kg.

Reference ID: 3903201

The dose normalized AUC and C_{max} and the 90% CI of MB in plasma are within the bioequivalence bounds. However, the 90% CI of dose normalized C_{max} of MB in whole blood is outside the bioequivalence bounds (**Table 8**). Similarly, the 90% CI of the dose normalized C_{max} of AZ in whole blood falls outside the bioequivalence bounds and the AZ plasma PK parameters are outside the bioequivalence bounds.

Table 8: Parallel BE study Parameters for MB and Azure B (AZ) in Whole Blood and Plasma

Analyte	Parameter	GMR	90% CI
MB Whole Blood	AUC_{inf}/D	104	96-113
	C_{max}/D	**115**	**96-137**
AZ Whole Blood	AUC_{inf}/D	94	81-110
	C_{max}/D	**53**	**44-63**
MB Plasma	AUC_{inf}/D	103	96-112
	C_{max}/D	87	82-96
AZ Plasma	AUC_{inf}/D	**74**	**68-81**
	C_{max}/D	**47**	**44-52**

The higher C_{max} of (b) (4) in whole blood is likely due to the concentration-dependent partitioning (see **Figure 4**). (b) (4)

(b) (4)

(b) (4)

2.5.1.2 If the formulation does not meet the standard criteria for bioequivalence, what clinical pharmacology and/or safety and efficacy data support the approval of the to-be-marketed product?

The difference in dose normalized C_{max} is likely due to differences in the administered dose and not due to inherent differences of test and reference products. Since the slight C_{max} difference is due to dosing considerations, the BE results do not indicate clinically meaningful differences between the two products.

2.6 Analytical Section

2.6.1 How are the active moieties identified and measured in the plasma?

The parent drug and metabolite (Azure B) were measured in plasma and whole blood.

2.6.2 Which metabolites have been selected for analysis and why?

Azure B was selected for analysis. Azure B was found to be the only metabolite detected in the metabolite screening study in human hepatocytes in vitro.

2.6.3 For all moieties measured, is free, bound, or total measured?

Total concentrations were measured for both MB and Azure B.

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2.6.4 What bioanalytical methods are used to assess concentrations?

The concentration of MB and Azure B were determined in whole blood and plasma using a liquid chromatography tandem mass spectrometry assay.

2.6.4.1 What is the range of the standard curve? How does it relate to the requirements for clinical studies? What is curve fitting technique?

The standard curve spanned a range of concentrations from 20 ng/mL to 5000 ng/mL and 10 ng/mL to 1000 ng/mL for MB and Azure B in whole blood and plasma (**Tables 9 and 10**). The concentration ranges covered the observed C_{max} (~3000 ng/mL for MB and 133 ng/mL for Azure B) and lowest observed concentration at 72 hours (40 ng/mL for MB and 13 ng/mL for Azure B). Weighted (1/x2) linear regression was used for back calculation of MB and Azure B concentrations.

2.6.4.2 What are the lower and upper limits of quantitation?

The LLOQ was 20 ng/mL and 10 ng/mL for MB and Azure B. The ULOQ was 5000 ng/mL and 1000 ng/mL for MB and Azure B.

2.6.4.3 What are the accuracy, precision, and selectivity at these limits?

The within-run and between-run precision and accuracy for MB and AZ in whole blood and plasma did not exceed 12%.

2.6.4.4 What is the sample stability under conditions used in the study?

2.6.4.5 The benchtop stability of MB and Azure B in whole blood and plasma was determined to be at least 24 hours under conditions used in the study (room temperature). The precision and accuracy were within the 15% limit.What is the QC sample plan?

Duplicate QC samples at 30 ng/mL (low), 350 ng/mL (medium), and 4000 ng/mL (high) for MB were included in each run. For Azure B, duplicate QC samples were included at 30 ng/mL (low), 125 ng/mL (medium), and 800 ng/mL (high). The accuracy and precision of the QC samples were well below the 15% limit for the accepted runs.

Reference ID: 3903201

Table 9: Methylene Blue Method Validation Summary

Analyte	Methylene blue
Internal Standard	Methylene blue - (b) (4)
Calibration range	10.0 - 5000 ng/mL
Regression type	Linear, weighted ($1/x^2$)
Blank matrix evaluation	(b) (4)
Matrix Effect	(b) (4)
Mutual interference methylene blue and methylene blue- (b) (4)	
Carryover Evaluation	
Within-run Precision (%CV) and Accuracy (%Bias)	(b) (4)
Between-run Precision (%CV) and Accuracy (%Bias)	
Recovery	(b) (4)
Bench-top Stability	
Re-injection Stability	
Freeze/thaw Stability	
Stock Solution Stability	
Spike Solution Stability	
Whole Blood Stability	
Long-term Frozen Sample Storage Stability	
Dilution Integrity	
Batch Size Determination	

Reference ID: 3903201

Table 10: Azure B Method Validation Summary

Analyte	Azure B
Internal Standard	Azure B- (b)
Calibration range	10.0 - 1000 ng/mL
Regression type	Linear, weighted ($1/x^2$)
Blank matrix evaluation	(b) (4)
Matrix Effect	
Mutual interference azure B and azure B- (b) (4)	
Carryover Evaluation	
Within-run Precision (%CV) and Accuracy (%Bias)	(b) (4)
Between-run Precision (%CV) and Accuracy (%Bias)	
Recovery	(b) (4)
Bench-top Stability	
Re-injection Stability	
Freeze/thaw Stability	
Stock Solution Stability	
Spike Solution Stability	
Whole Blood Stability	
Long-term Frozen Sample Storage Stability	
Dilution Integrity	
Batch Size Determination	

Reference ID: 3903201

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/s/

SALAHELDIN S HAMED
03/17/2016

BAHRU A HABTEMARIAM
03/18/2016

NAM ATIQUR RAHMAN
03/18/2016
I agree with the review team's recommendation.

Office of Clinical Pharmacology Review

Date of Submission:	07/18/2014
Application Number (SDN):	IND 118156 (007) and NDA 204630 (043)
Product Name:	(b) (4)® (Methylene Blue Injection 0.5%)
Route of Administration:	Intravenous administration
Sponsor:	Provepharm SAS
Submission Type:	Response to Agency Comments

This review outlines a response to comments sent to the sponsor on 7/14/14 regarding new relative BA/BE protocol for (b) (4) (Methylene Blue Injection 0.5%). This drug is being developed for the treatment of acquired methemoglobinemia ((b) (4) . This drug is also being developed under IND 113942. A 505(b)(2) NDA application using literature to support the proposed indication is currently being reviewed under NDA 204630. A detailed description of the background for this issue and pharmacokinetics of (b) (4) and methylene blue injection USP 1% (MB 1%) can be found in the 5/29/14 and 7/14/14 clinical pharmacology reviews for this application.

Sponsor responses to Clinical Pharmacology Related Comments

FDA Comment #1 [7/14/2014]: Given you have not provided information regarding the partitioning of methylene blue into red blood cells, which is its site of action, and many trials in the literature have reported methylene blue PK in whole blood, you should justify your decision to collect plasma samples.

> **Sponsor response [7/18/2014]:** The use of plasma samples was decided based on literature reference Walter-Sack 2009. The authors analyzed methylene blue (MB) concentration in plasma and whole blood after IV administration of MB 1% and concluded that plasma and whole blood MB concentrations did not differ when only MB was administered alone, indicating that MB does not accumulate within a cellular compartment, such as the erythrocytes.
>
> Provepharm and Luitpold do not currently have a method for the analysis of MB or azure B in whole blood. The development and validation of the analytical method are expected to take at least one month.
>
> In addition, the comparator MB 1% product to be used in the study will be sourced from Luitpold Pharmaceuticals, Inc. (American Reagent). Provepharm has previously submitted to NDA 20-4630 the report from the completed Luitpold PK study 1MBL2002 (A One-Period, Single-Dose, Safety, Tolerability, and Pharmacokinetic Study of Methylene Blue Injection USP Following a 1 mg/kg Intravenous Dose in Healthy Adult Volunteers). Since plasma concentrations for MB and azure B (metabolite) were analyzed in this study, the collection of plasma samples in the new PK study allows the use of the same, validated analytical method. It would also allow comparison with the results from the previously completed Luitpold PK study.
>
> **FDA Comment: Your response is noted. Your justification for using plasma samples should be included in your final trial report when it is submitted to the Agency.**

FDA Comment #2 [7/14/2014]: We note in your response to our previous comments that you intend to use the validated methods that were used for determination of the concentrations of methylene blue and azure B for the Luitpold Pharmaceuticals Inc. 1MBL12002 protocol. You should cross validate these assays to rule out any potential effect that (b) (4) differences between (b) (4) and methylene blue injection USP 1% may have on them.

Sponsor response [7/18/2014]: The validated assays are not affected by [redacted] as they are validated to measure the amount of either methylene blue or azure B present in a sample. The [redacted] of the drug product should not affect the validity of the assay but may affect the levels of each seen at different time points. Provepharm is unclear as to how to proceed with this issue and request additional information if this is in fact required.

FDA Comment: Your response is noted. To address the Agency's concerns we recommend that you validate and utilize two assay methods. The first should be used exclusively for [redacted] and all standards, QC samples, and calibrations associated with the assay should use the [redacted] product. The second should be used exclusively for methylene blue injection USP 1% and all standards, QC samples and calibrations should use the methylene blue injection USP 1% product.

Recommendation:

The Office of Clinical Pharmacology, Division of Clinical Pharmacology V, has reviewed the sponsor's responses to the Agency's 7/14/2014 comments from a clinical pharmacology perspective and provided two comments that should be communicated the sponsor.

Signatures:

Joseph A. Grillo, Pharm.D.
Reviewer
Division of Clinical Pharmacology 5

Julie Bullock, Pharm.D.
Team Leader
Division of Clinical Pharmacology 5

Cc: DDOP: CSO – **K Robertson;** MTL – **A Deisseroth**; MO – **D Przepiorka**
DCP-5: Reviewer - **J Grillo**; TL - **J Bullock**; Deputy DD - **B Booth**; DD - **A Rahman**

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/s/

JOSEPH A GRILLO
08/14/2014

JULIE M BULLOCK
08/15/2014

Office of Clinical Pharmacology Review

Date of Submission:	06/20/14
Application Number (SDN):	IND 118156 (005) and NDA 204630 (039)
Product Name:	[redacted] (b) (4)® (Methylene Blue Injection 0.5%)
Route of Administration:	Intravenous administration
Sponsor:	Provepharm SAS
Submission Type:	New BA/BE Protocol

This review outlines new relative BA/BE protocol [redacted] (b) (4) (Methylene Blue Injection 0.5%) for the treatment of acquired methemoglobinemia ([redacted] (b) (4)). This drug is also being developed under IND 113942. A 505(b)(2) NDA application using literature to support the proposed indication is currently being reviewed under NDA 204630.

Background: While methylene blue products (i.e., methylene blue injection USP 1% (10 mg/mL)) solution) are commercialized in the USA, there are currently no methylene blue products approved by the FDA or listed in the Orange Book as a reference product (active or discontinued). Therefore, there is no reference listed product.

[redacted] (b) (4) Injection, 0.5% is not the same concentration as USP Methylene Blue (1%) that is currently used clinically as an unlicensed pharmaceutical. The sponsor states that the [redacted] (b) (4) compared with other currently available Methylene Blue USP products. [redacted] (b) (4)

Despite this the sponsor is requesting a BA biowaiver in the current NDA application under review by arguing that bioavailability of this intravenous product is self-evident.

As part of its ongoing review of the NDA application, the Agency finds that it cannot grant the biowaiver because, as stated above, there is no listed drug to reference for the waiver. The Agency is also unable to link BA/BE to the literature because the PK information provided by the sponsor for methylene blue injection USP 1% (10 mg/mL) is highly variable due to a combination of assay, formulation and sampling issues (Table 1). The most comprehensive information is from the Luitpold 2013 trial which was conducted in healthy volunteers by another company, with right of reference to the sponsor, using the American Reagent's (i.e., Luitpold Pharmaceuticals, Inc.) methylene blue injection USP 1% (10 mg/mL). Unfortunately the results from this PK trial are deemed inconclusive because the results are not similar to any of the available published PK literature.

Table 1: Summary of Clinical Pharmacology Studies

Trial	Source	Dose	N	Medium	Sampling (hr)	C_{max} (ug/ml)		AUC (µg/mL*hr)		CL	$t_{1/2}$
						Observed	Normalized (100 mg)	Observed	Normalized (100 mg)		
Peter et al [2000] [1]	compounded	100 mg	7	Blood	4	3	3.0	5.0	5.0	3.0	5.2
Repici et al [2012][2]	commercial	100 mg	22	Blood	72	2.07	2.1	13.5	13.5	7.4	26.7
Walter-Sack et al [2008] [3]	compounded	50 mg	15	Blood	24	1.4	2.8	6.5	12.9	7.7	13.6
			10	Plasma	24	0.75	1.5	7.6	15.2	6.6	18.5
Luitpold [2013]#	commercial	1 mg/kg	12	Plasma	72	0.49	1.0	3.1	6.1	19.5	17.5

Right of reference submitted to NDA 204630

Source: Reviewer adapted from information in NDA 204630 clinical pharmacology summary and literature references

[1] Peter C, Hongwan D, Küpfer A, Lauterburg BH. Pharmacokinetics and organ distribution of intravenous and oral methylene blue. *Eur J Clin Pharmacol.* 2000 Jun;56(3):247-50.

[2] Repici A1, Di Stefano AF, Radicioni MM, Jas V, Moro L, Danese S. Methylene blue MMX tablets for chromoendoscopy. Safety tolerability and bioavailability in healthy volunteers. *Contemp Clin Trials.* 2012 Mar;33(2):260-7.

[3] Walter-Sack I, Rengelshausen J, Oberwittler H, Burhenne J, Mueller O, Meissner P, Mikus G. High absolute bioavailability of methylene blue given as an aqueous oral formulation. *Eur J Clin Pharmacol.* 2009 Feb;65(2):179-89.

The Agency held a teleconference with the sponsor on April 30, 2014 to resolve issues identified from the Division's Midcycle for its NDA application that are highlighted above. The main outcome from the meeting was that the sponsor would conduct a comparative PK study of Provepharm's methylene blue drug product compared to another commercialized USP Methylene Blue (1%) product available in the US. (b) (4)

[Reviewer comment: (b) (4)

Previous Relevant Regulatory History:

- 2/6/12 PreNDA meeting (IND 113,942) (b) (4) 2) Literature submitted is not sufficient to support NDA because conflicting results will not permit a regulatory determination without additional confirmatory information (i.e., ADME trial with PK analysis), 3) additional detail required regarding the in vitro CYP inhibition and induction studies, and 4) Potential for QTc prolongation and potential to inhibit the P-gp transporter system were not adequately addressed.
- 10/11/12 Refuse to File (RTF) (NDA 204630): 1) format of proposed labeling inadequate, 2) QTc prolongation potential not addressed, 3) Provide detailed study reports for in vitro studies, 4) Provide organ impairment studies, and 4) comments from 2/6/12 letter reiterated.
- 5/16/13 IRT Consult: 1) Data provided will not address the potential of your drug to prolong the QT/QTc interval because the ECGs are not available for the patients treated in France and the ECGs collected in the Cosmo Pharmaceuticals clinical study are collected at a time (Day 5) when systemic concentration of methylene blue is minimal or nonexistent.
- 5/20/13 Response to RTF: 1) agreed with IRT assessment, 2) Additional information from published trials does not resolve concerns regarding the variability in PK data and trial to characterize PK using validated assay continues to be recommended.
- 5/29/14 Protocol synopsis review: 1) cannot provide definitive comments without complete protocol, 2) Concomitant sensitive substrates with a narrow therapeutic range of CYP 1A2 , 2B6 , 2C9, and 2C19 should not be permitted, 3) Design trial as a single-dose, crossover rather than parallel or justify your rational, 4) An adequate washout period should separate each treatment, 5) Justify the proposed sample size, 6) employ an equivalence approach for BE comparisons that includes the calculation of a 90% confidence interval for the ratio of geometric means of the AUC and Cmax measures, and 7) Validate the analytical methods used to determine the concentrations of methylene blue and azure B.

Pharmacokinetic Parameters

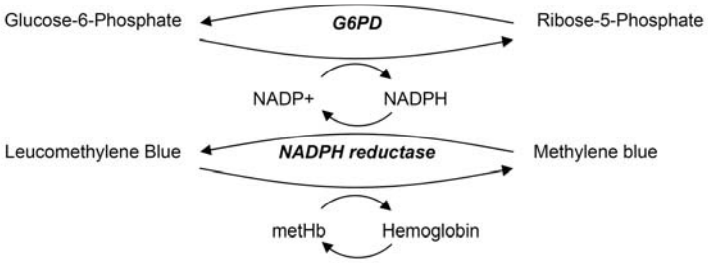
See background information and Table 1 above for an overview of available information regarding the human PK of intravenously administered methylene blue injection USP 1% (10 mg/mL).

Distribution: The in vitro protein binding in human plasma of (b) (4) and Methylene Blue USP, by equilibrium dialysis, was 94% and 97%, respectively. An Examination of autopsied peripheral organs of a patient receiving intravenous methylene blue have showed that the concentrations of methylene blue in these tissues were 74–208 ng/g while the concentrations of

Reference ID: 3592275

it metabolite azure B (475–2943 ng/g) were significantly higher.[4] This may be related to the ability for oxidized azure B to assume a neutral quinoneimine form that readily diffuses through membranes that is in contrast to oxidized methylene blue.[4]

Metabolism: In humans methylene blue is metabolized to yield the N-demethylated metabolites, azure B as major active metabolite and azure A as secondary metabolite. Under physiological conditions MB also undergoes redox cycling and is reduced to yield the uncharged species, leucomethylene blue which plays an important role in MB's effects in the treatment of methemoglobinemia (Figure 1). Certain types of hereditary glucose-6-phosphate dehydrogenase deficiency (G6PD deficiency) may affect methylene blue exposure because this enzyme provides antioxidant-reducing equivalents in the form of NADPH.



Source: *Clinical pharmacology summary NDA 204630*

Figure 1: Reduction of methemoglobin to hemoglobin by NADPH reductase

Elimination: Based on animal studies methylene blue is eliminated in bile, feces, and urine as parent and leukomethylene blue. In one literature report, a mean of 29±3% of the administered dose was eliminated in the urine over 24 hours following intravenous bolus administration of methylene blue 100 mg to seven volunteers.[1] Leucomethylene blue was approximately one third of the total urinary recovery. Following oral administration of methylene blue 10mg to seven volunteers, a mean of 74% (53-97%) of the administered dose was eliminated in the urine over 5 days. On average, 78% of the total urine recovery was leucomethylene blue (65%-85%).[5]

Drug Interaction (DDI) potential: The sponsor reports both products had very similar DDI potential. The sponsor reports that in vitro (assuming Cmax =0.75 µg/mL or 2.34 µmol [MW: 319.85]) its product and methylene blue injection USP 1% (10 mg/mL) are potent inhibitors (CYP (IC_{50})) of CYP 1A2 (<0.05), 2B6 (2.4-2.7), 2C9 (1-1.3) and 2C19 (<0.05). The sponsor also reports that its product and methylene blue injection USP 1% (10 mg/mL) does not induce CYP enzymes 1A2 and 3A4; however this is indeterminate due to the poor affect seen with the positive controls.

Methylene blue and it's metabolite Azure B are inhibitors of MAO and concurrent use with medicinal products that enhance serotonergic transmission including SSRIs (selective serotonin reuptake inhibitors), bupropion, buspirone, clomipramine, mirtazapine and venlafaxine should be avoided.[6]

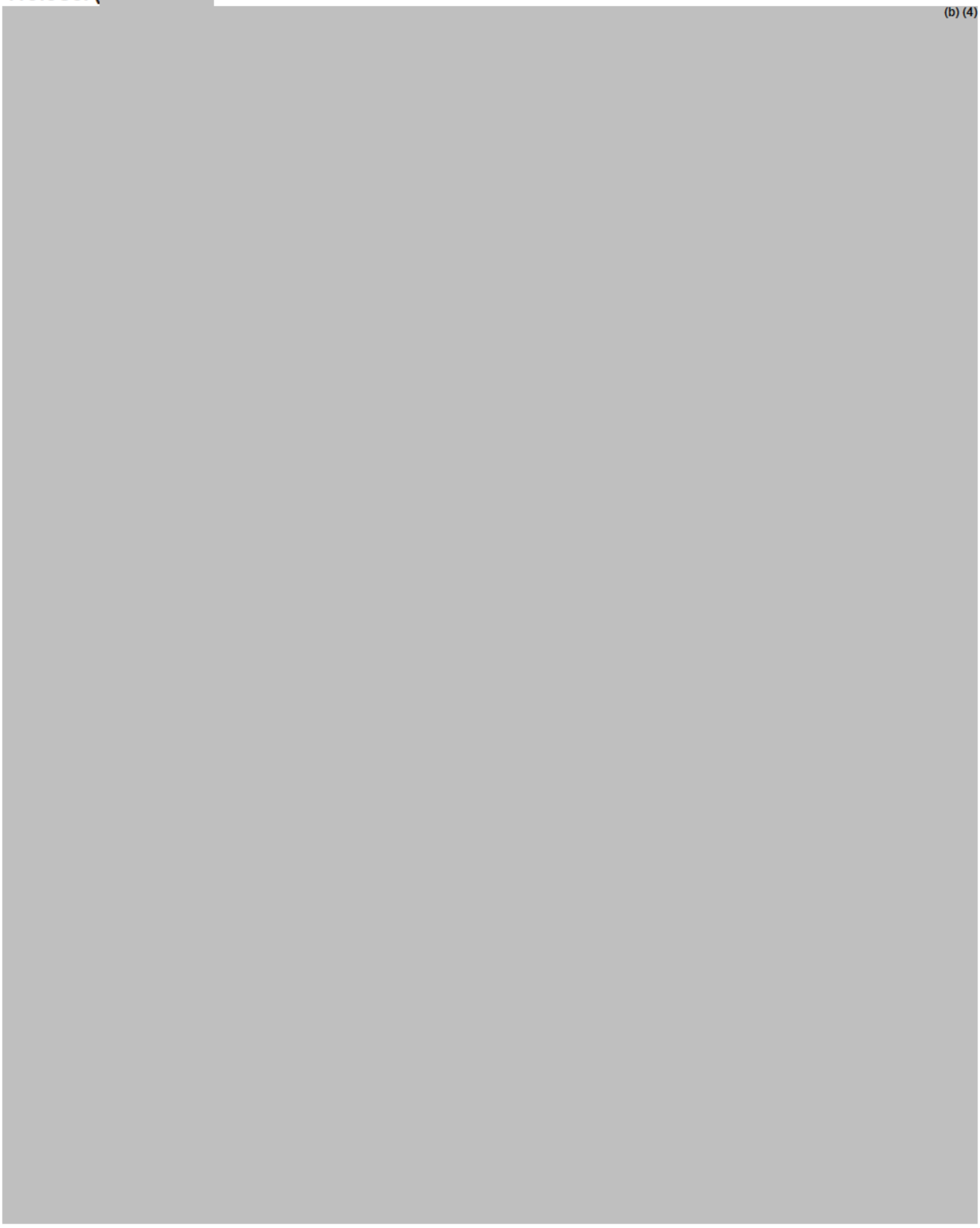
Pediatrics: Vetrella[7] et al. report that erythrocytic enzymes show different ontogenetic changes. NADPH-MR has significantly higher activity in the erythrocytes of prematures, newborns and infants than in older children and adults. The activity of NADPH-MR decreases significantly until

[4] Petzer A1, Harvey BH, Wegener G, Petzer JP. Azure B, a metabolite of methylene blue, is a high-potency, reversible inhibitor of monoamine oxidase. *Toxicol Appl Pharmacol.* 2012 Feb 1;258(3):403-9.
[5] DiSanto AR, Wagner JG. Pharmacokinetics of highly ionized drugs. II. Methylene blue--absorption, metabolism, and excretion in man and dog after oral administration. *J Pharm Sci.* 1972 Jul;61(7):1086-90.
[6] Ramsay RR1, Dunford C, Gillman PK. Methylene blue and serotonin toxicity: inhibition of monoamine oxidase A (MAO A) confirms a theoretical prediction. *Br J Pharmacol.* 2007 Nov;152(6):946-51
[7] Vetrella M, Astedt B, Barthelmai W, Neuvians D. Activity of NADH- and NADPH-dependent methemoglobin reductases in erythrocytes from fetal to adult age. A parallel assessment. *Klin Wochenschr.* 1971 Sep 1;49(17):972-7.

Reference ID: 3592275

the age between 7 months and 3 years and thereafter increases again to adult values: (comparison of the age group 7 months to 3 years vs. adults: $p < 0.01$).

Protocol ((b) (4)



(b) (4)

Pharmacodynamics: None

Clinical Pharmacology Comments

1) Given you have not provided information regarding the partitioning of methylene blue into red blood cells, which is its site of action, and many trials in the literature have reported methylene blue PK in whole blood, you should justify your decision to collect plasma samples.

2) We note in your response to our previous comments that you intend to use the validated methods that were used for determination of the concentrations of methylene blue and azure B for the Luitpold Pharmaceuticals Inc. 1MBL12002 protocol. You should cross validate these assays to rule out any potential effect that the solubility differences between (b) (4) and methylene blue injection USP 1% may have on them.

Recommendation:

The Office of Clinical Pharmacology, Division of Clinical Pharmacology V, has reviewed this protocol from a clinical pharmacology perspective and provided two comments that should be communicated the sponsor.

Signatures:

Joseph A. Grillo, Pharm.D.
Reviewer
Division of Clinical Pharmacology 5

Julie Bullock, Pharm.D.
Team Leader
Division of Clinical Pharmacology 5

Cc: DDOP: CSO – **K Robertson;** MTL – **A Deisseroth**; MO – **D Przepiorka**
DCP-5: Reviewer - **J Grillo**; TL - **J Bullock**; Deputy DD - **B Booth**; DD - **A Rahman**

Reference ID: 3592275

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/s/

JOSEPH A GRILLO
07/14/2014

JULIE M BULLOCK
07/14/2014

Office of Clinical Pharmacology Review

Date of Submission:	05/16/14
Application Number (SDN):	IND 118156 (004)
Product Name:	[redacted]® (Methylene Blue Injection 0.5%)
Route of Administration:	Intravenous administration
Sponsor:	Provepharm SAS
Submission Type:	New BA/BE Protocol Synopsis

This review outlines new relative BA/BE protocol synopsis [redacted] (Methylene Blue Injection 0.5%) for the treatment of acquired methemoglobinemia ([redacted]. This drug is also being developed under IND 113942. A 505(b)(2) NDA application using literature to support the proposed indication is currently being reviewed under NDA 204630.

Background: While methylene blue products (i.e., methylene blue injection USP 1% (10 mg/mL)) solution) are commercialized in the USA, there are currently no methylene blue products approved by the FDA or listed in the Orange Book as a reference product (active or discontinued). Therefore, there is no reference listed product.

[redacted] Injection, 0.5% is not the same concentration as USP Methylene Blue (1%) that is currently used clinically as an unlicensed pharmaceutical. The sponsor states that the [redacted] compared with other currently available Methylene Blue USP products [redacted]
Despite this the sponsor is requesting a BA biowaiver in the current NDA application under review by arguing that bioavailability of this intravenous product is self-evident.

As part of its ongoing review of the NDA application, the Agnecy finds that it cannot grant the biowaiver because, as stated above, there is no listed drug to reference for the waiver. The Agency is also unable to link BA/BE to the literature because the PK information provided by the sponsor for methylene blue injection USP 1% (10 mg/mL) is highly variable due to a combination of assay, formulation and sampling issues (Table 1). The most comprehensive information is from the Luitpold 2013 trial which was conducted in healthy volunteers by another company, with right of reference to the sponsor, using the American Reagent's (i.e., Luitpold Pharmaceuticals, Inc.) methylene blue injection USP 1% (10 mg/mL). Unfortunately the results from this PK trial are deemed inconclusive because the results are not similar to any of the available published PK literature. The Agency is currently inspecting the clinical and analytical sites to look for potential confounding factors (e.g., sample collection, handling and storage) that may have contributed to these findings.

Table 1: Summary of Clinical Pharmacology Studies

Trial	Source	Dose	N	Medium	Sampling (hr)	C_{max} (ug/ml)		AUC (µg/mL*hr)		CL	$t_{1/2}$
						Observed	Normalized (100 mg)	Observed	Normalized (100 mg)		
Peter et al [2000] [1]	compounded	100 mg	7	Blood	4	3	3.0	5.0	5.0	3.0	5.2
Repici et al [2012][2]	commercial	100 mg	22	Blood	72	2.07	2.1	13.5	13.5	7.4	26.7
Walter-Sack et al [2008] [3]	compounded	50 mg	15	Blood	24	1.4	2.8	6.5	12.9	7.7	13.6
			10	Plasma	24	0.75	1.5	7.6	15.2	6.6	18.5
Luitpold [2013][#]	commercial	1 mg/kg	12	Plasma	72	0.49	1.0	3.1	6.1	19.5	17.5

Write of reference submitted to NDA 204630

Source: Reviewer adapted from information in NDA 204630 clinical pharmacology summary and literature references

The Agency held a teleconference with the sponsor on April 30, 2014 to resolve issues identified from the Division's Midcycle for its NDA application that are highlighted above. The main outcome from the meeting was that the sponsor would conduct a comparative PK study of Provepharm's methylene blue drug product compared to another commercialized USP Methylene Blue (1%) product available in the US. (b) (4)

[Reviewer comment: (b) (4)

Previous Relevant Regulatory History:

- 2/6/12 PreNDA meeting (IND 113,942):1) (b) (4) , 2) Literature submitted is not sufficient to support NDA because conflicting results will not permit a regulatory determination without additional confirmatory information (i.e., ADME trial with PK analysis), 3) additional detail required regarding the in vitro CYP inhibition and induction studies, and 4) Potential for QTc prolongation and potential to inhibit the P-gp transporter system were not adequately addressed.
- 10/11/12 Refuse to File (RTF) (NDA 204630): 1) format of proposed labeling inadequate, 2) QTc prolongation potential not addressed, 3) Provide detailed study reports for in vitro studies, 4) Provide organ impairment studies, and 4) comments from 2/6/12 letter reiterated.
- 5/16/13 IRT Consult: 1) Data provided will not address the potential of your drug to prolong the QT/QTc interval because the ECGs are not available for the patients treated in France and the ECGs collected in the Cosmo Pharmaceuticals clinical study are collected at a time (Day 5) when systemic concentration of methylene blue is minimal or nonexistent.
- 5/20/13 Response to RTF: 1) agreed with IRT assessment, 2) Additional information from published trials does not resolve concerns regarding the variability in PK data and trial to characterize PK using validated assay continues to be recommended.

Pharmacokinetic Parameters

See background information and Table 1 above for an overview of available information regarding the human PK of intravenously administered methylene blue injection USP 1% (10 mg/mL).

[1] Peter C, Hongwan D, Küpfer A, Lauterburg BH. Pharmacokinetics and organ distribution of intravenous and oral methylene blue. *Eur J Clin Pharmacol.* 2000 Jun;56(3):247-50.

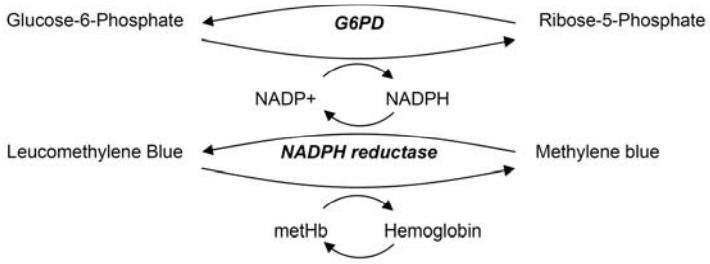
[2] Repici A1, Di Stefano AF, Radicioni MM, Jas V, Moro L, Danese S. Methylene blue MMX tablets for chromoendoscopy. Safety tolerability and bioavailability in healthy volunteers. *Contemp Clin Trials.* 2012 Mar;33(2):260-7.

[3] Walter-Sack I, Rengelshausen J, Oberwittler H, Burhenne J, Mueller O, Meissner P, Mikus G. High absolute bioavailability of methylene blue given as an aqueous oral formulation. *Eur J Clin Pharmacol.* 2009 Feb;65(2):179-89.

Reference ID: 3512935

Distribution: The in vitro protein binding in human plasma of [redacted] (b) (4) and Methylene Blue USP, by equilibrium dialysis, was 94% and 97%, respectively. An Examination of autopsied peripheral organs of a patient receiving intravenous methylene blue have showed that the concentrations of methylene blue in these tissues were 74–208 ng/g while the concentrations of it metabolite azure B (475–2943 ng/g) were significantly higher. [4] This may be related to the ability for oxidized azure B to assume a neutral quinoneimine form that readily diffuses through membranes that is in contrast to oxidized methylene blue.[4]

Metabolism: In humans methylene blue is metabolized to yield the N-demethylated metabolites, azure B as major active metabolite and azure A as secondary metabolite. Under physiological conditions MB also undergoes redox cycling and is reduced to yield the uncharged species, leucomethylene blue which is key to its effects in the treatment of methemoglobinemia (Figure 1). Certain types of hereditary glucose-6-phosphate dehydrogenase deficiency (G6PD deficiency) may affect methylene blue exposure because this enzyme provides antioxidant-reducing equivalents in the form of NADPH.



Source: Clinical pharmacology summary NDA 204630

Figure 1: Reduction of methemoglobin to hemoglobin by NADPH reductase

Elimination: Based on animal studies methylene blue is eliminated in bile, feces, and urine as parent and leukomethylene blue. In one literature report, a mean of 29±3% of the administered dose was eliminated in the urine over 24 hours following intravenous bolus administration of methylene blue 100 mg to seven volunteers.[1] Leucomethylene blue was approximately one third of the total urinary recovery. Following oral administration of methylene blue 10mg to seven volunteers, a mean of 74% (53-97%) of the administered dose was eliminated in the urine over 5 days. On average, 78% of the total urine recovery was leucomethylene blue (65%-85%).[5]

Drug Interaction (DDI) potential: The sponsor reports both products had very similar DDI potential. The sponsor reports that in vitro (assuming Cmax =0.75 µg/mL or 2.34 µmol [MW: 319.85]) its product and methylene blue injection USP 1% (10 mg/mL) are potent inhibitors (CYP (IC_{50})) of CYP 1A2 (<0.05), 2B6 (2.4-2.7), 2C9 (1-1.3) and 2C19 (<0.05) and an inducer of 1A2 and 3A4. The later induction information is not included in the referenced NDA 204630 in vitro report and cannot be confirmed. Methylene blue and it's metabolite Azure B are inhibitors of MAO and concurrent use with medicinal products that enhance serotonergic transmission including SSRIs (selective serotonin reuptake inhibitors), bupropion, buspirone, clomipramine, mirtazapine and venlafaxine.[6]

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[4] Petzer A1, Harvey BH, Wegener G, Petzer JP. Azure B, a metabolite of methylene blue, is a high-potency, reversible inhibitor of monoamine oxidase. *Toxicol Appl Pharmacol.* 2012 Feb 1;258(3):403-9.

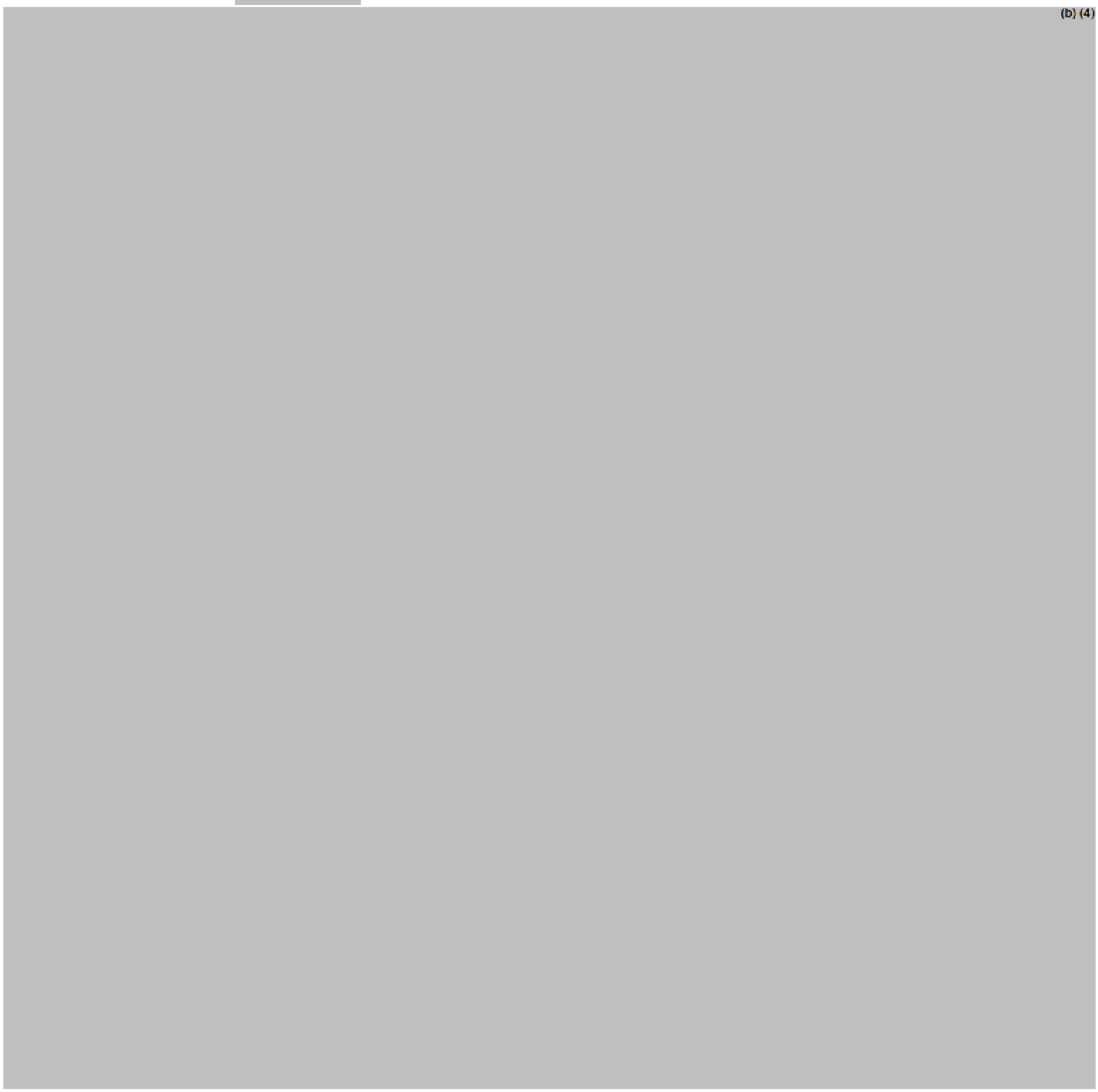
[5] DiSanto AR, Wagner JG. Pharmacokinetics of highly ionized drugs. II. Methylene blue--absorption, metabolism, and excretion in man and dog after oral administration. *J Pharm Sci.* 1972 Jul;61(7):1086-90.

[6] Ramsay RR1, Dunford C, Gillman PK. Methylene blue and serotonin toxicity: inhibition of monoamine oxidase A (MAO A) confirms a theoretical prediction. *Br J Pharmacol.* 2007 Nov;152(6):946-51

Reference ID: 3512935

Pediatrics: Vetrella[7] et al. report that erythrocytic enzymes show different ontogenetic changes. NADPH-MR has significantly higher activity in the erythrocytes of prematures, newborns and infants than in older children and adults. The activity of NADPH-MR decreases significantly until the age between 7 months and 3 years and thereafter increases again to adult values: (comparison of the age group 7 months to 3 years vs. adults: $p < 0.01$).

Protocol Synopsis (b) (4)



[7] Vetrella M, Astedt B, Barthelmai W, Neuvians D. Activity of NADH- and NADPH-dependent methemoglobin reductases in erythrocytes from fetal to adult age. A parallel assessment. *Klin Wochenschr.* 1971 Sep 1;49(17):972-7.

Clinical Pharmacology Comments

1) We cannot provide definitive comments until you submit a complete protocol. The following preliminary comments are based on the protocol synopsis that you provided:

   a) Concomitant use of drugs that are sensitive in vivo substrates and/or CYP substrates with a narrow therapeutic range (Refer to the *Drug Interaction Studies* Guidance for more information)of CYP 1A2 , 2B6 , 2C9, and 2C19 should not be permitted prior to or during the proposed trial. A comprehensive list of agents should be included as an appendix in the final protocol.

   b) Design your trial as a single-dose, crossover (b) (4) An adequate washout period (e.g., more than 5 half-lives of the moieties to be measured) should separate each treatment in a cross over design. Refer to the Guidance for Industry Statistical Approaches to Establishing Bioequivalence.

   c) Justify that the proposed sample size is adequate to power your proposed trial. Refer to the Guidance for Industry Statistical Approaches to Establishing Bioequivalence.

   d) Employ an equivalence approach for BE comparisons that includes the calculation of a 90% confidence interval for the ratio of geometric means of the AUC and Cmax measures for (b) (4) and methylene blue injection USP 1% (10 mg/mL) solution. A BE limit of 80 to 125% for the ratio of the product averages has should be adopted. Refer to the Guidance for Industry Statistical Approaches to Establishing Bioequivalence.

   e) Validate the analytical methods used to determine the concentrations of methylene blue and azure B. Refer to the Guidance for Industry *Bioanalytical Method Validation*.

Recommendation:

- The Office of Clinical Pharmacology, Division of Clinical Pharmacology V, has reviewed this meeting package from a clinical pharmacology perspective and provided five comments that should be communicated the sponsor.

Signatures:

Joseph A. Grillo, Pharm.D.
Reviewer
Division of Clinical Pharmacology 5

Julie Bullock, Pharm.D.
Team Leader
Division of Clinical Pharmacology 5

Cc: DDOP: CSO – **K Robertson;** MTL – **A Deisseroth**; MO – **D Przepiorka**
DCP-5: Reviewer - **J Grillo**; TL - **J Bullock**; Deputy DD - **B Booth**; DD - **A Rahman**

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/s/

JOSEPH A GRILLO
05/27/2014

JULIE M BULLOCK
05/29/2014

BIOPHARMACEUTICS REVIEW Office of New Drug Quality Assessment			
Application No.:	NDA 204-630	**Reviewer:** Minerva Hughes, Ph.D.	
Submission Date:	13 September 2013 (resubmission)		
Division:	Division of Hematology Products	**Team Leader:** Angelica Dorantes, Ph.D.	
Sponsor:	Provepharm SAS	**Secondary Reviewer:** Elsbeth Chikhale, Ph.D. (Acting Team Lead)	
Trade Name:	(b) (4) Injection	**Date Assigned:**	20 September 2013
		GRMP Date: **PDUFA Date:**	8 Sept 2014 13 Oct 2014
Generic Name:	Methylene blue	**Date of Review:**	2 Sept 2014
Indication:	Methemoglobinemia	**Type of Submission:** Original 505(b)2 Resubmission after RTF Marketed Unapproved Drug	
Dosage form/strengths	Solution for Injection (0.5%)		
Route of Administration	Intravenous		
Overall Conclusion/ Recommendation: Request to waive the BA/BE study requirement is *denied*.			

SUBMISSION SUMMARY

NDA 204-630 was submitted as a 505(b)2 application for the use of (b) (4)® Injection, 0.5%, methylene blue (MB; methylthioninium chloride) in the treatment of acquired methemoglobinemia. The intended dose is 1 (b) (4) mg/kg (b) (4) given intravenously over a period of 5 minutes. There are no methylene blue products approved in the US or listed in the Orange Book as a reference product. The Applicant submits this 505(b)(2) NDA using literature to support the proposed indication, but does not have a reference listed product for the application. This NDA submission is a resubmission of the initial application submitted on 13 August 2012, which was refused to file on 11 October 2012 citing a lack of data to support the efficacy and safety claims.

The proposed drug product is a 10 mL solution for injection formulation of the methylene blue drug substance and water. Although not approved in the US, there are marketed methylene blue injection products in the United Kingdom and the United States (USP monograph); however, all of the formulations are formulated as a 1% w/v injection solution. The drug substance used by the Applicant is manufactured (b) (4)

The Applicant requests a waiver of the regulatory requirement to submit bioavailability (BA)

and bioequivalence (BE) data for the NDA product, referencing 21 CFR 320.22. However, as noted in the 12 Nov 2013 Quality Filing review by this Reviewer (see DARRTS), a biowaiver request under 21 CFR 320.22 is not applicable for this product because there is no approved product to reference. A meeting was held on 30 April 2014 to discuss the BA/BE deficiency with the Applicant and the Applicant has agreed to conduct a clinical pharmacokinetic study to address this deficiency, which will be evaluated by the Clinical Pharmacology Reviewer. This review provides an overview of the Applicant's justification and reiterates the Biopharmaceutics decision to deny the requested waiver.

BIOPHARMACEUTIC INFORMATION/BIOWAIVER REQUEST REVIEW

As per 21 CFR 320.22, the in vivo BA or BE of a drug product may be self-evident and the requirement for BA or BE data waived if the drug product meets the following criteria. The required criteria referenced and the Applicant's information are summarized below.

1. The drug product is a parenteral solution intended solely for administration by injection, or an ophthalmic or otic solution; and

Applicant's Information: The drug product is intended only for intravenous administration

2. Contains the same active and inactive ingredients in the same concentration as a drug product that is the subject of an approved full new drug application or abbreviated new drug application

Applicant's Information: The Applicant acknowledges that there are no approved methylene blue products in the U.S. Further, (b) (4) injection, 0.5%, is not the same concentration as the commercial USP (1%) products and does not meet this regulatory requirement. However, because the product is an injection product, the Applicant believes that it still qualifies for a waiver. The difference in concentration is (b) (4) for the (b) (4) drug substance. The firm has supplied the following supportive information to evaluate whether the (b) (4) and reduced concentration may affect bioavailability.

- A plasma protein binding study (see Clinical Pharmacology Review)
- An inhibition of cytochromes P450 study (see Clinical Pharmacology Review)
- Physicochemical characterization relative to commercial USP products. (see CMC/Quality Review)

In addition, the Applicant submitted the clinical study report for PK study AA98923, a Luitpold study using a 1% methylene blue product manufactured by Luitpold Pharmaceuticals, to address FDA's comments in the RTF letter regarding the inadequacy of the PK information in the submitted literature studies.

Based on these data, the Applicant believes that the comparability of the PK profile, and thus the clinical efficacy and safety is demonstrated.

Reference ID: 3622251

Reviewer's Assessment: Not acceptable. A waiver of the BA/BE study requirement is not applicable for this product given the lack of an available listed drug to serve as a basis for safety and efficacy. Consequently, the NDA must be supported with adequate BA data, which may be submitted through literature or clinical studies. However, reliance on literature studies must be scientifically appropriate. In this case, adequate literature data on methylene blue PK is lacking, as the PK results conflict among the reports (see FDA communications under IND 113942 and Clinical Pharmacology Review for IND 113942 dated 6 February 2012 by Dr. Joseph Grillo). Further, the scientific rationale for reliance on a 1% product, manufactured by a different supplier, (b) (4) remains unclear, (b) (4) (to be addressed by the Clinical Pharmacology Reviewer). The Applicant has agreed to conduct a clinical PK study using the NDA product to address the FDA's concerns. The clinical protocol and study report review will be addressed by the Clinical Pharmacology Reviewer. There are no additional issues for Biopharmaceutics.

CONCLUSION/RECOMMENDATION: The Applicant's request for a waiver of the BA/BE study requirement is NOT GRANTED because (1) there is no listed drug to serve as a basis for safety and efficacy and (2) an acceptable bridge to the products used in the literature was not demonstrated. To address this deficiency, the Applicant has agreed to conduct a clinical PK study, which will be evaluated by the Clinical Pharmacology Reviewer. Therefore, the final recommendation on the NDA approvability with respect to the adequacy of the BA/BE data is deferred to the Office of Clinical Pharmacology.

Minerva Hughes, Ph.D.
Biopharmaceutics Reviewer
Office of New Drug Quality Assessment

Elsbeth Chikhale, Ph.D.
Biopharmaceutics Team Leader (Acting)
Office of New Drug Quality Assessment

Reference ID: 3622251

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/s/

MINERVA HUGHES
09/05/2014

ELSBETH G CHIKHALE
09/05/2014

OFFICE OF CLINICAL PHARMACOLOGY REVIEW

NDA: 204630	**Submission Dates (SDN):** 9/13/2013 (9); 11/12/2013 (14); 11/19/13 (16); 11/25/13 (17); 1/7/14 (20); 4/17/14 (25); 5/7/14 (29); 5/20/14 (31); 6/12/14 (35); 6/18/14 (38); 6/20/14 (39); 6/27/14 (41); 7/18/14 (43)
Brand Name:	[redacted] (b) (4) Injection, 0.5%
Generic Name:	Methylene Blue Injection, 0.5%
Reviewer:	Joseph Grillo, Pharm.D.
Team Leader:	Julie Bullock, Pharm.D.
OCP Division:	DCP V
OND Division:	OHOP\Division of Hematology Products (DHP)
Sponsor:	Provepharm SAS
Relevant IND(s):	113,942 and 118156
Submission Type; Code:	NDA (Section 505(b)(2))
Formulation; Strength(s):	0.5% sterile solution of methylene blue for intravenous administration. 10 mL ampule
Indication:	Antidote indicated for the treatment of acquired methemoglobinemia [redacted] (b) (4)

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1 EXECUTIVE SUMMARY

This application is an NDA for [redacted] Injection, 0.5% [redacted] submitted under section 505(b)(2) of the Federal Food, Drug, and Cosmetic Act (the Act). The applicant did not conduct any clinical pharmacology trials using proposed to-be-marketed [redacted] injection, 0.5% [redacted] The submission includes literature data to support the safety and effectiveness of [redacted] injection. The pharmacokinetic (PK) as well as the majority of the safety and efficacy data submitted in this NDA were generated after administration of methylene blue injection USP 1% (MB 1% USP). The applicant also performed a small (n=12) retrospective clinical trial in patients who were treated for methemoglobinemia with foreign sourced [redacted] in France and the United Kingdom to support safety and efficacy.

The Office of New Drug Quality Assessment (ONDQA) could not grant a biowaiver from determining the relative Bioavailability/Bioequivalence (BA/BE) between MB 1% USP used in the majority of the clinical trials and [redacted] because there is no listed reference drug to support the waiver. The Agency is also unable to establish BA/BE by linking the submitted published and unpublished MB 1% USP PK literature to [redacted] because the PK information provided in these papers is highly variable due to a likely combination of assay, formulation, and sampling issues. In the absence of relative BA/BE between [redacted] and MB 1% USP, a link to extrapolate from the MB 1% USP based retrospective trials and case reports supporting safety and efficacy cannot be established. In addition, the lack of any dose-concentration-response or ontogeny information for [redacted] or MB 1% USP does not allow for a formal assessment of the adult dose and [redacted] pediatric dose. Furthermore, the applicant did not provide mass balance information to adequately describe the metabolic and elimination pathways for either [redacted] or MB 1% USP.

Therefore, this application fails to provide sufficient information to support a recommendation of approval of [redacted] due to the absence of relative BA/BE between [redacted] Injection, 0.5% and methylene blue injection USP 1%. Additional deficiencies include; 1) lack of information regarding the pharmacokinetics of [redacted] and the ADME of methylene blue (MB) in general, 2) highly variable MB 1% USP pharmacokinetic (PK) information in published and unpublished reports, and 3) the inability to assess the proposed [redacted] dose in adults, pediatrics, and special populations (e.g., renal impairment).

1.1 Recommendation

The Office of Clinical Pharmacology (OCP) has determined that there is insufficient clinical pharmacology and biopharmaceutics information provided in this NDA to support a recommendation of approval of [redacted] We recommend a complete response (CR) action until the relative bioequivalence between [redacted] injection 0.5% (5 mg/mL) solution and methylene blue injection USP 1% (10 mg/mL) solution is established. The applicant should also address the deficiencies below in their resubmission of the application. The acceptability of specific drug information is provided below.

Reference ID: 3621071

Decision	Acceptable to OCP?			Comment
Overall	Yes ☐	No ☒	NA ☐	Deficient due to combination of the absence of relative BA/BE, highly variable PK and the inability to assess the proposed dosing regimen.
Evidence of Effectiveness†	Yes ☐	No ☒	NA ☐	Cannot establish a link to extrapolate from the MB 1% USP based clinical trials and reports to support safety and efficacy to (b) (4)
Proposed dose for general population	Yes ☐	No ☒	NA ☐	Dose concentration response or ontogeny information to allow for a formal assessment of dose not provided.
Proposed dose selection for others	Yes ☐	No ☒	NA ☐	Inadequate ADME information provided.
Pivotal BE	Yes ☐	No ☒	NA ☐	A biowaiver cannot be granted because there is no listed drug. Cannot link BA/BE to the submitted published and unpublished MB 1% USP PK reports because of high variability.
Labeling	Yes ☐	No ☒	NA ☐	The above issues limit the safe labeling of this drug from a clinical pharmacology perspective.

†This decision is from a clinical pharmacology perspective only. The overall safety and effectiveness determination is made by the Clinical reviewer.

1.2 Deficiencies

1.2.1 Conduct a relative BA/BE trial in humans to assess the relative bioequivalence of methylene blue and Azure B concentrations following intravenous administration of (b) (4) injection 0.5% (5 mg/mL) solution or methylene blue injection USP 1% (10 mg/mL) solution . Samples should be analyzed using a validated method (Refer to the Guidance for Industry Bioanalytical Method Validation) and full PK profiling from both methylene blue formulations should be reported for both methylene blue and Azure B.

1.2.2 Identify the pathways by which (b) (4) (and its metabolites) are eliminated and excreted in humans.

1.2.3 (b) (4)

1.2.4 Conduct a clinical trial in humans to determine the potential of (b) (4) to prolong the QT/QTc interval according to the principles of ICH E14.

1.2.5 Evaluate the in vitro partitioning of (b) (4) and its metabolite Azure B into human red blood cells.

1.2.6 Evaluate the in vitro ability of (b) (4) (and its metabolites) to act as substrates of cytochrome P450 enzymes, was well as substrates or inhibitors of conjugating enzymes and transporters. For an updated list of potential metabolic pathways and transporters identified by the Agency please refer to the newly released draft guidance *Drug Interaction Studies*.

1.3 Post Marketing Requirements

Not applicable

1.4 Post Marketing Commitments

Not applicable

1.5 Comments to the Applicants

Not applicable

1.6 Summary of Important Clinical Pharmacology and Biopharmaceutics Findings

This application is an NDA for [redacted] that was submitted under section 505(b)(2) of the Act. This application fails to provide sufficient information to support a recommendation of approval of [redacted] due to the absence of relative BA/BE between [redacted] Injection, 0.5% and methylene blue injection USP 1%. Additional deficiencies are listed above and described below.

[redacted] is a sterile solution for intravenous administration with the proposed indication as an antidote for the treatment of acquired methemoglobinemia [redacted] in adult and pediatric patients. [redacted] is not the same concentration as MB 1% USP that is currently used clinically as an unlicensed pharmaceutical. [redacted] While methylene blue products (i.e., MB 1% USP) are commercialized in the USA, there are currently no MB products approved by the FDA or listed in the Orange Book as a reference product (active or discontinued). Therefore, there is no listed drug to serve as the basis for a biowaiver.

The Agency is also unable to establish BA/BE by linking to the literature because the published and unpublished PK information provided by the applicant for MB 1% USP is highly variable (i.e., dose normalized (DN) Cmax: 1 to 5 µg/ml, DN AUC: 5 to 15.2 µg/ml*hr, clearance (CL): 3 to 19.5 L/hr., and half-life ($t_{1/2}$): 5.2 to 26.7 hrs) due to a combination of assay, formulation and sampling issues. The most comprehensive information is from a trial in healthy volunteers by another company, with right of reference to the applicant, using MB 1% USP. Unfortunately, the results from this PK trial are inconclusive because the results are not similar to any of the available published MB 1% USP PK information. The applicant did not provide any information regarding the clinical PK of [redacted]

Also without relative BA/BE between [redacted] and MB 1% USP, a link to extrapolate from the MB 1% USP based retrospective trials and case reports supporting safety and efficacy cannot be established for [redacted] from a clinical pharmacology (CP) perspective. These issues combined with the lack of any dose concentration response or ontogeny information for [redacted] or MB 1% USP does not allow for a formal assessment of the adult dose and [redacted] pediatric dose. Therefore, the applicant should conduct a relative BA/BE trial comparing [redacted] to MB 1% USP as well as the PK of methylene blue and Azure B in each formulation.

The in vitro protein binding in human plasma of [redacted] and MB 1% USP, by equilibrium dialysis, was 94% and 97%, respectively. Under physiological conditions MB undergoes redox cycling and is reduced to yield the uncharged species, leucomethylene blue which plays an important role in MB's effects in the treatment of methemoglobinemia. In humans methylene blue is also likely metabolized by an unidentified pathway to yield the N-demethylated metabolites, azure B as major active metabolite and azure A as secondary metabolite.

In one report from the published literature, a mean of 29±3% of the administered dose was eliminated in the urine over 24 hours following intravenous bolus administration of MB 1% USP to a small population of healthy volunteers. The complete elimination profile of methylene blue and Azure B are unknown and should be evaluated by the applicant. [redacted] Avoidance in patients with moderate to severe renal or hepatic disease unless the potential benefit justifies the potential risk and close monitoring for patients

with any degree of impairment is recommended until additional clinical information can be generated in these special populations.

In vitro [redacted] (b) (4) and MB 1% USP are inhibitors of CYP 1A2, 2B6, 2C9 and 2C19. [redacted] (b) (4) Additional in vivo drug interaction trials are not recommended at this time given the acute nature of this therapy. Methylene blue and its metabolite Azure B are likely inhibitors of Monoamine Oxidase (MAO) and concurrent use with medicinal products that enhance serotonergic transmission including SSRIs (selective serotonin reuptake inhibitors), bupropion, buspirone, clomipramine, mirtazapine and venlafaxine should be avoided.

Signatures

Joseph Grillo, Pharm.D.
Clinical Pharmacology Reviewer
Division of Clinical Pharmacology 5

Julie Bullock, Pharm.D.
Clinical Pharmacology Team Leader
Division of Clinical Pharmacology 5

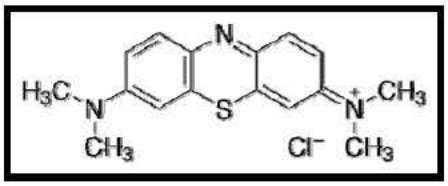
Nam Atiqur Rahman, Ph.D.
Division Director
Division of Clinical Pharmacology 5

2 QUESTION BASED REVIEW

2.1 General Attributes

2.1.1 What are the highlights of the chemistry and physical-chemical properties of the drug substance and the formulation of the drug product as they relate to clinical pharmacology and biopharmaceutics review?

Methylene blue is a water-soluble thiazine dye. The molecular formula of MB is $C_{16}H_{18}CLN_3S$ and its molecular weight is 319.86 g/mol. Its structural formula is:



(b) (4) is a sterile solution intended for intravenous administration. Each (b) (4) Injection, 0.5%, 10 mL ampule contains 50 mg (b) (4) methylene blue and water for injection (q.s) resulting in each 1 mL of solution containing 5 mg methylene (b) (4) Injection, 0.5% is a clear dark blue solution with a pH value between 3.0 and 4.5. The osmolality is between 10 and 15 mOsm/kg.

(b) (4) is not the same concentration as MB 1% USP that is currently used clinically as an unlicensed pharmaceutical. The applicant states that the manufacturing process of (b) (4) (b) (4) The applicant has not provided adequate justification to substantiate this comparison and we defer to the Drug Quality Assessment (ONDQA) reviewer. Further, the applicant has not provided adequate justification to evaluate the potential impact of these (b) (4) differences on the clinical pharmacology, biopharmaceutics, efficacy, and safety of methylene blue therapy.

2.1.2 What are the proposed mechanism of action and therapeutic indication?

The applicant is proposing that (b) (4) be indicated as an antidote indicated for the treatment of acquired methemoglobinemia ((b) (4) (b) (4)). This indication includes both adult and pediatric patients.

The mechanism of action of MB for the proposed indication (Figure 1) (b) (4) In turn, LMB promotes a non-enzymatic redox reaction of Methemoglobin (metHb) to hemoglobin. Since MB is itself an oxidizer, at higher concentration it is thought to have a paradoxical effect and promote metHb generation.

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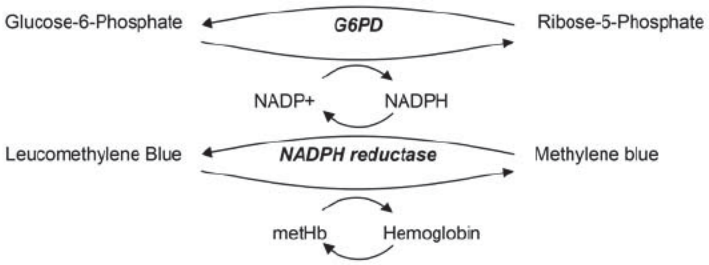

Source: *Clinical pharmacology summary NDA 204630*

Figure 1: Reduction of methemoglobin to hemoglobin by NADPH reductase

2.1.3 What are the proposed dosage(s) and route(s) of administration?

(b) (4) is administered intravenously as a slow bolus injection over a period of 5 minutes or diluted in 5% dextrose in water (D5W). The applicant proposes the dosing regimen described in below.

Table 1: Proposed (b) (4) dosing

(b) (4)

Source: *Applicant's proposed labeling*

2.2 General Clinical Pharmacology

2.2.1 What are the design features of the clinical pharmacology and clinical studies used to support dosing or claims?

The applicant did not conduct any clinical pharmacology related trials using (b) (4) The applicant submitted three open label PK trials from the medical literature and one unpublished trial from another company with right of reference that used intravenously administered MB 1% USP.

To support proposed efficacy claims the applicant performed a small (n=12) retrospective clinical trial (PVP-2013001) in patients at "as many hospitals as possible" in France and the United Kingdom who were treated for methemoglobinemia with foreign sourced (b) (4) and asked them to provide all clinical information regarding patients (age from 0-11 years (n=3, 25%) and 18- 65 years (n=9, 75%)) that were treated. In addition, the applicant conducted a comprehensive medical literature search of reported cases of methemoglobinemia treated by all MB products (primarily MB 1% USP). This gleaned four small retrospective trials in addition to numerous case reports.

2.2.2 What is the basis for selecting the response endpoints (i.e., clinical or surrogate endpoints) or biomarkers (collectively called pharmacodynamics (PD)) and how are they measured in clinical pharmacology and clinical studies?

The primary efficacy variable in the applicant's retrospective trial PVP-2013001 was percent methemoglobin level of blood which is acceptable. The four retrospective trials and numerous case reports used varying endpoints such as symptomatic improvement, arterial blood gases, and methemoglobin levels which are also reasonable response endpoints from a clinical pharmacology perspective.

2.2.3 Are the active moieties in the plasma (or other biological fluid) appropriately identified and measured to assess pharmacokinetic parameters and exposure response relationships?

The MB metabolite Azure B may contribute to the neuro- and psychomodulatory effects of MB and also may act as a potential inhibitor of monoamine oxidase (MAO).[1] [2] The applicant has not provided any information regarding exposure and PK of Azure B following intravenous administration of (b) (4) The applicant provided information regarding the exposure and PK of Azure B following intravenously administered MB 1% USP that was conducted by another company with right of reference but, these findings cannot be extrapolated to (b) (4) due to the lack of relative BA/BE between (b) (4) and MB 1% USP (see Section 2.5.2).

2.2.4 Exposure-response

2.2.4.1 What are the characteristics of the exposure-response relationships (dose-response, concentration-response) for efficacy? If relevant, indicate the time to the onset and offset of the desirable pharmacological response or clinical endpoint.

The applicant has not provided any efficacy related exposure response information for (b) (4) or MB 1% USP.

2.2.4.2 What are the characteristics of the exposure-response relationships (dose-response, concentration-response) for safety? If relevant, indicate the time to the onset and offset of the undesirable pharmacological response or clinical endpoint.

The applicant has not provided any safety related exposure response information for (b) (4) or MB 1% USP.

2.2.4.3 Does this drug prolong the QT or QTc interval? (You must answer this question, unless this is addressed in the question above.)

The effect of (b) (4) on the QT or QTc interval has not been determined. In response to a deficiency in a 10/11/2012 refuse to file letter for the proposed indication, the applicant submitted cardiac monitoring in 10 patients in France treated for methemoglobinemia with (b) (4) and a clinical study[3] to evaluate in healthy volunteers the safety and the bioavailability in 22 patients with (b) (4) in

[1] Ramsay RR, Dunford C, Gillman PK. Methylene blue and serotonin toxicity: inhibition of monoamine oxidase A (MAO A) confirms a theoretical prediction. *Br J Pharmacol.* 2007 Nov;152(6):946-51

[2] Petzer A, Harvey BH, Wegener G, Petzer JP. Azure B, a metabolite of methylene blue, is a high-potency, reversible inhibitor of monoamine oxidase. *Toxicol Appl Pharmacol.* 2012 Feb 1;258(3):403-9.

[3] Repici A, Di Stafano AFD, Radicioni MM, Jas V, Danese S .. Methylene blue MMX® tablets for chromoendoscopy. Safety tolerability and bioavailability in healthy volunteers. *Contemporary Clinical Trials* 2012; 33:260-267.

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MMX tablets (b) (4) compared to intravenously administered MB 1% USP on 3/28/2013. The QT Interdisciplinary Review Team (QT-IRT) reviewed this information on 5/16/2013 and determined that these data will not address the potential of (b) (4) to prolong the QT/QTc interval because the ECGs are not available for the patients treated in France and the ECGs collected in the Cosmo Pharmaceuticals clinical study are collected at a time (Day 5) when systemic concentration of methylene blue is minimal or nonexistent.

In the current application the applicant submits information from a study from another company with right of reference (CSR No AA98923). In this PK trial, conducted with MB injection 1% USP, 12-lead ECGs were performed on all the patients (12) at screening, before dosing on Day 1 and at 0.25, 1, 4 and 24 hrs post-dose, and at the end of the study or upon early termination. The actual ECG tracings were not provided.

QT-IRT analysis (see 6/30/2014 QT-IRT review) of these data show the point estimate and the 90% CIs corresponding to the largest upper bounds for for ΔQTcF (ms) following a single dose of MB injection 1% USP (1 mg/kg) was 7.6 ms (-7.4 ms ~ 22.6 ms) at 7 minutes post dose. Based on this analysis QT-IRT finds that a large QTc prolongation (> 20 ms) effect of 1 mg/kg methylene blue injection could not be ruled out in this study because the largest upper bound of the 2-sided 90% CI for the mean change from baseline was 22.6 ms observed at 7 minutes postdose. Furthermore, the QT effect is difficult to interpret in this study because of the following reasons: 1) The study did not include a positive control (moxifloxacin) arm (i.e., no assay sensitivity was established) or a supratherapeutic dose, 2) ECG measurements were done sparsely (i.e., only one measurement around Tmax), 3) The large upper bound of ΔQTcF could be an artifact of small sample size and lack of time-matched baseline values, and 4) The concentration-ΔQTcF analysis did not show a positive relationship of ΔQTcF to concentrations. Therefore the QT-IRT concludes that a thorough QT (TQT) study should be conducted.

The OCP reviewer agrees with the conclusions and recommendations of the QT-IRT and adds that these findings cannot be extrapolated to (b) (4) due to the lack of relative BA/BE between (b) (4) and MB 1% USP (see Section 2.5.2). The potential of (b) (4) to prolong the QT/QTc interval is still a deficiency requiring the completion of a QT study according to the principles of ICH E14.

2.2.4.4 Is the dose and dosing regimen selected by the sponsor consistent with the known relationship between dose-concentration-response, and are there any unresolved dosing or administration issues?

The applicant did not provide dose concentration response information for (b) (4) or MB 1% USP to allow for a formal assessment of dose. The proposed dosing recommendations are based on safety and efficacy endpoints from the applicant's uncontrolled small retrospective trial using (b) (4) as well as other small uncontrolled retrospective trials and case reports from the medical literature that primarily used MB 1% USP. Due to the absence of PK/PD information we defer to the Clinical reviewer regarding whether the observed safety and efficacy profile from the submitted trial and case study information justify the proposed dose in adult and pediatric patients being treated for the proposed indication. We caution against extrapolation of findings from MB 1% USP to (b) (4) due to the lack of relative BA/BE between these products (see Section 2.5.2). This lack of relative BA/BE is a deficiency requiring the completion of a trial to assess this issue.

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2.2.5 What are the PK characteristics of the drug and its major metabolite?

The applicant did not provide information regarding the PK characteristics for (b) (4) The applicant submitted three single dose open label PK trials from the medical literature and one unpublished trial from another company with right of reference that provided information regarding the PK characteristics of intravenously administered MB 1% USP (see Table 2). We caution against extrapolation of findings from MB 1% USP to (b) (4) due to the lack of relative BA/BE between these products (see Section 2.5.2).

In addition, the results from these submitted MB 1% USP based PK trials are highly variable (see Table 2). A cross trial visual evaluation of this variability focusing on differences in plasma vs. blood sampling, PK sampling time, formulation, and assay, by the Agency was unable to resolve the source of the variability. Further, the most comprehensive information is from the "Luitpold 2013" trial which was conducted in healthy volunteers by another company, with right of reference to the applicant, using the American Reagent's (i.e., a subsidiary of Luitpold Pharmaceuticals, Inc.) MB 1% USP; however, these results (Table 2 and Table 3) were not similar to any of the submitted published PK trials. The Agency is currently inspecting the clinical and analytical sites to look for potential confounding factors (e.g., sample collection, handling and storage) that may have contributed to these findings. The results of this inspection are pending. Therefore, these submitted published and unpublished trials describing the PK characteristics of MB and the metabolite Azure B following intravenous administration of MB 1% USP are considered indeterminate due to the variability between the trials that is likely the result of a combination of assay, formulation and sampling issues. It is important to note that these concerns regarding (b) (4) were communicated to the applicant prior to this submission on 2/6/12 (IND 113,942), 10/11/12, and 5/20/13. The applicant should conduct a trial to describe the PK characteristics of (b) (4) and its metabolites. This can be incorporated into the needed relative BA/BE between (b) (4) and MB 1% USP discussed earlier.

Table 2: Summary of Clinical Pharmacology Studies with intravenously administered MB 1% USP

Trial	Source	Dose	N	Medium	Sampling (hr)	C_{max} (ug/ml)		AUC (µg/mL*hr)		CL (L/Hr)	$t_{1/2}$ (Hrs)
						Observed	Normalized (100 mg)	Observed	Normalized (100 mg)		
Peter et al [2000] [4]	compounded	100 mg	7	Blood	4	3	3.0	5.0	5.0	3.0	5.2
Repici et al [2012][3]	commercial	100 mg	22	Blood	72	2.07	2.1	13.5	13.5	7.4	26.7
Walter-Sack et al [2008] [5]	compounded	50 mg	15	Blood	24	1.4	2.8	6.5	12.9	7.7	13.6
			10	Plasma	24	0.75	1.5	7.6	15.2	6.6	18.5
Luitpold [2013][#]	commercial	1 mg/kg	12	Plasma	72	0.49	1.0	3.1	6.1	19.5	17.5

Write of reference submitted to NDA 204630

Source: Reviewer adapted from information in NDA 204630 clinical pharmacology summary and literature references

[4] Peter C, Hongwan D, Küpfer A, Lauterburg BH. Pharmacokinetics and organ distribution of intravenous and oral methylene blue. *Eur J Clin Pharmacol.* 2000 Jun;56(3):247-50.

[5] Walter-Sack I, Rengelshausen J, Oberwittler H, Burhenne J, Mueller O, Meissner P, Mikus G. High absolute bioavailability of methylene blue given as an aqueous oral formulation. *Eur J Clin Pharmacol.* 2009 Feb;65(2):179-89.

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Table 3: Summary of the Mean (SD) Plasma Methylene Blue and Azure B Pharmacokinetic Parameters following intravenous administration of a 1 mg/kg dose of MB 1% USP

Pharmacokinetic Parameters	Methylene Blue	Azure B
AUC_{0-t} (ng*hr/mL)	2692.91 (725.913)	504.05 (169.490)
$AUC_{0-\infty}$ (ng*hr/mL)	3069.42 (826.486)	717.99 (203.939)
$AUC_{\%extrap}$ (%)	12.28 (3.976)	27.70 (4.135)
CL (L/hr)	19.469 (4.5264)	N/A
Cmax (ng/mL)	492.333 (198.1851)	85.858 (26.5550)
tmax # (hr)	0.19 (0.08, 0.75)	0.53 (0.08, 1.00)
$t_{1/2}$ (hr)	17.468 (8.4658)	11.214 (4.3056)
λ_z (1/hr)	0.04734 (0.019294)	0.07287 (0.033287)
V_z (L)	467.158 (177.7640)	N/A

For tmax, median (minimum, maximum) are presented
N/A = Not applicable
Source: Applicant's report for trial 1MBL12002 (right of reference submitted)

2.2.5.1 What are the single dose and multiple dose PK parameters?

See Section 2.2.5 regarding single intravenous dose PK of MB 1% USP. Information regarding single dose PK following intravenous administration of (b) (4) or multiple dose PK for either (b) (4) or MB 1% USP was not provided by the applicant.

2.2.5.2 How does the PK of the drug and its major active metabolites in healthy volunteers compare to that in patients?

This information was not provided by the applicant for either (b) (4) or MB 1% USP.

2.2.5.3 What are the characteristics of drug absorption?

Not applicable to this intravenously administered formulation.

2.2.5.4 What are the characteristics of drug distribution?

The applicant did not provide volume of distribution information for (b) (4) Based on Cl and t1/2 reported in Table 2 the volume of distribution (Vd) of MB 1% USP is estimated (Vd=(t1/2*Cl)/0.693)to be 22 L to 285 L from whole blood sampling and 176L to 492 L from plasma sampling. The Vd of the metabolite Azure B was not reported. These findings are considered inconclusive given the high variability as discussed in Section 2.2.5. Further, these findings cannot be extrapolated to (b) (4) due to the lack of relative BA/BE between (b) (4) and MB 1% USP (see Section 2.5.2).

The applicant assessed the in vitro protein binding of both (b) (4) and MB 1% USP using equilibrium dialysis and reported it to be 94% and 97%, respectively. These findings are considered inconclusive given the low recovery (i.e., 50% and 64%, respectively) from the in vitro system (b) (4) The applicant should also assess MB red cell partitioning given that this is the target of MB therapy and the variability in MB PK seen in Table 2.

An examination of autopsied peripheral organs of a patient receiving intravenous methylene blue in the published literature reports that the concentrations of methylene blue in these tissues were substantially lower than its metabolite azure B.[2] This may be related to the ability for oxidized azure B to assume a neutral

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quinoneimine form that readily diffuses through membranes that is in contrast to oxidized methylene blue; however insufficient information is available to make a conclusive determination at this time.[7]

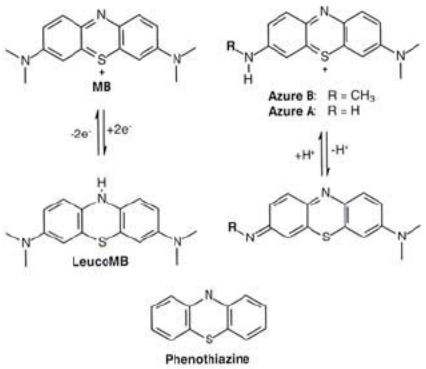
2.2.5.5 Does the mass balance study suggest renal or hepatic as the major route of elimination?

The applicant did not provide complete human mass balance information for [redacted] (b) (4) or MB 1% USP. In one literature report, a mean of 29±3% of a 100 mg MB 1% USP intravenous bolus dose was eliminated in the urine over 24 hours in seven volunteers.[4] LMB was approximately one third of the total urinary recovery in this trial. These findings are considered inconclusive given the high variability in the submitted PK trials discussed in Section 2.2.5. Further, these findings cannot be extrapolated to [redacted] (b) (4) due to the lack of relative BA/BE between [redacted] (b) (4) and MB 1% USP (see Section 2.5.2).

2.2.5.6 What are the characteristics of drug metabolism?

As discussed in Section 2.1.2, MB undergoes redox cycling and is reduced to yield the uncharged species LMB under physiological conditions.[6] At least one report suggests that the erythrocytic enzymes responsible for this redox cycling may show different ontogenetic changes.[7] NADPH reductase has significantly higher activity in the erythrocytes of prematures, newborns and infants compared to older children and adults. The activity of NADPH reductase appears to decreases until the age between 7 months and 3 years and thereafter increases again to adult values.

In humans MB is also metabolized to yield the N-demethylated metabolites, azure B as major metabolite and azure A as secondary metabolite.[4] [8] The specific enzymes responsible for this N-demethylation in humans is not known. [redacted] (b) (4). The applicant should investigate the metabolic pathways of MB in vitro.



Source: *Reference #2*

Figure 2: Known metabolic pathways of methylene blue

[6] K Buchholz, R. Heiner Schirmer, JK Eubel, MB. Akoachere, T Dandekar, K Becker, and S Gromer. Interactions of Methylene Blue with Human Disulfide Reductases and Their Orthologues from Plasmodium falciparum. *Antimicrob Agents Chemother.* Jan 2008; 52(1): 183–191.
[7] Vetrella M, Astedt B, Barthelmai W, Neuvians D. Activity of NADH- and NADPH-dependent methemoglobin reductases in erythrocytes from fetal to adult age. A parallel assessment. *Klin Wochenschr.* 1971 Sep 1;49(17):972-7.
[8] Warth A, Goeppert B, Bopp C, Schirmacher P, Flechtenmacher C, Burhenne J. Turquoise to dark green organs at autopsy. *Virchows Arch.* 2009 Mar;454(3):341-4.

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2.2.5.7 What are the characteristics of drug excretion?

The applicant did not provide excretion information for (b) (4) The CL and $t_{1/2}$ reported in Table 2 for MB 1% USP is 3 to 7.7 L/hr and 5.2 to 26.7 hrs from whole blood sampling and 6.6 to 19.5 L/hr and 17.5 to 18.5 hrs from plasma sampling, respectively. The t 1/2 of the metabolite azure B following intravenous administration of MB 1% USP was reported to be approximately 11 hours. Azure B Cl was not reported. These findings are considered inconclusive given the high variability as discussed in Section 0. Further, these findings cannot be extrapolated to (b) (4) due to the lack of relative BA/BE between (b) (4) and MB 1% USP.

2.2.5.8 Based on PK parameters, what is the degree of linearity or nonlinearity in the dose-concentration relationship?

The degree of linearity or nonlinearity for (b) (4) or MB 1% USP is not reported by the applicant.

2.2.5.9 How do the PK parameters change with time following chronic dosing?

Not applicable. This is an acute therapy.

2.2.5.10 What is the inter- and intra-subject variability of PK parameters in volunteers and patients, and what are the major causes of variability?

The variability in MB CL from the unpublished normal healthy volunteer trial submitted with right of reference and published literature reports submitted to describe the PK of MB 1% USP was approximately 40% following whole blood sampling and 70% following plasma sampling. The within subject variability for the normal healthy volunteer trial submitted with right of reference for MB 1% USP was 30%.

2.3 Intrinsic Factors

2.3.1 What intrinsic factors (age, gender, race, weight, height, disease, genetic polymorphism, pregnancy, and organ dysfunction) influence exposure (PK usually) and/or response, and what is the impact of any differences in exposure on efficacy or safety responses?

The applicant did not provide any information regarding the impact of intrinsic factors on (b) (4) or MB 1% USP exposure. Based on the limited information provided in Section 2.2.5 the exposure of MB and its metabolites may potentially be affected by age (advanced age secondary to an age related decline in renal function and pediatrics), renal impairment, and hepatic impairment.

2.3.2 Based upon what is known about exposure-response relationships and their variability and the groups studied, healthy volunteers vs. patients vs. specific populations, what dosage regimen adjustments, if any, are recommended for each of these groups? If dosage regimen adjustments are not based upon exposure-response relationships, describe the alternative basis for the recommendation.

Information regarding exposure-response relationships for (b) (4) or MB 1% USP were not submitted. No specific dose modifications can be justified from the limited information provided by the applicant regarding the PK of (b) (4) or MB 1% USP.

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2.3.2.1 Elderly

The applicant did not provide any information regarding the impact of advanced age on (b) (4) or MB 1% USP exposure. Based on the limited information provided in Section 2.2.5 the exposure of MB and its metabolites may potentially be affected by advanced age secondary to an age related decline in renal function. Frequent monitoring should be recommended in labeling.

2.3.2.2 Pediatric patients

The applicant did not provide dose-concentration-response information for (b) (4) or MB 1% USP in pediatrics to allow for a formal assessment of dose. (b) (4) (b) (4) (b) (4) .

The proposed dosing recommendations are based solely on safety and efficacy endpoints in pediatric patients from the applicant's uncontrolled small retrospective trial using (b) (4) as well as other small uncontrolled retrospective trials and case reports from the medical literature that primarily used MB 1% USP. Due to the absence of PK/PD information in pediatric patients we defer to the Clinical reviewer regarding whether the observed safety and efficacy profile from the submitted trial and case study information justify the proposed dose in adult and pediatric patients being treated for the proposed indication. We caution against extrapolation of findings from MB 1% USP to (b) (4) due to the lack of relative BA/BE between these products (see Section 2.5.2).

2.3.2.3 Renal impairment

The applicant did not provide any information regarding the impact of renal impairment on MB or Azure B exposure following intravenous administration of (b) (4) or MB 1% USP. Based on the limited information provided in Section 2.2.5 the exposure of MB and its metabolites may potentially be affected by renal impairment but the magnitude of the effect cannot be definitively quantified. A small (n=7) study of healthy volunteers receiving a 100 mg intravenous bolus dose of MB 1% USP in the medical literature reports at least one third of the administered dose was eliminated renally.

(b) (4) Avoidance in patients with moderate to severe renal disease unless the potential benefit justifies the potential risk and frequent monitoring for patients with any degree of renal impairment is recommended.

[9] US Food and Drug Administration. Pharmacokinetics in Patients with Impaired Renal Function — Study Design, Data Analysis, and Impact on Dosing and Labeling. Draft issued 3/22/2010.

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2.3.2.4 Hepatic impairment

The applicant did not provide any information or labeling recommendations related to the impact of hepatic impairment on MB or Azure B exposure following intravenous administration of (b) (4) or MB 1% USP. Reports in the medical literature suggest that methylene blue injection USP 1% likely undergoes some metabolism based on the recovery of at least two metabolites (see Section 2.2.5). Avoidance in patients with moderate to severe hepatic disease unless the potential benefit justifies the potential risk and frequent monitoring for patients with any degree of hepatic impairment is recommended.

2.3.2.5 Other human factors that are important to understanding the drug's efficacy and safety

No.

2.4 Extrinsic Factors

2.4.1 What extrinsic factors (drugs, herbal products, diet, smoking, and alcohol use) influence dose-exposure and/or -response and what is the impact of any differences in exposure on response?

Unknown. The applicant provided limited information regarding the metabolic and transporter systems that may affect MB. This is compounded by the lack of information on exposure response and exposure safety of MB.

2.4.1.1 Based upon what is known about exposure-response relationships and their variability, what dosage regimen adjustments, if any, do you recommend for each of these factors? If dosage regimen adjustments across factors are not based on the exposure-response relationships, describe the basis for the recommendation.

None (see Section 2.4.1).

2.4.2 Drug-drug interactions

2.4.2.1 Is there an in vitro basis to suspect in vivo drug-drug interactions?

Yes (see Section 2.4.2.3).

2.4.2.2 Is the drug a substrate of CYP enzymes? Is metabolism influenced by genetics?

Unknown. The applicant should determine the metabolic and transporter based pathways for (b) (4) in vitro and as part of the mass balance trial discussed earlier in this review.

2.4.2.3 Is the drug an inhibitor and/or an inducer of CYP enzymes?

CYP450 inhibition by (b) (4) or MB 1% USP was evaluated in vitro using recombinant individually expressed CYP450s for CYP 1A2, 2B6, 2C9, 2C19, 2D6, and 3A4. Positive controls consistent with the Agency DDI draft guidance we used for CYP 1A2, 2C9, 2D6, and 3A4. The applicant did not justify that the probe tranylcypromine was sufficiently sensitive to be an acceptable positive control for CYP 2B6 and 2C19. Based on the IC_{50} results of this study (Table 4) inhibition of CYPs 1A2, 2B6, 2C9, 2C19, and 3A4 is likely. The applicant did not provide an R value (1+([I]/Ki) for these CYPs so a more definitive analysis was not possible. Conclusions

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regarding the magnitude of the inhibition of CYP 2B6 and 2C19 by (b) (4) or MB 1% USP is limited given the concerns regarding the inadequate positive control used in the study. The potential for in vitro inhibition of 1A2, 2B6, 2C9, 2C19, and 3A4 should be communicated in labeling; however, the lack of information regarding the clinical relevance of these findings should also be noted. Given the acute and limited nature of the proposed use of intravenous (b) (4) for the proposed indication, additional in vivo studies are not required at this time. Defer to the Clinical reviewer regarding whether the theoretical impact of MB related CYP1A2 inhibition on intravenous caffeine exposure in neonates warrants cautionary language in the labeling or additional study given caffeine is thought to have a wider therapeutic index compared to theophylline.

Table 4: In vitro Inhibition of CYP Isoforms by MB 1% USP and (b) (4)

Compound	IC_{50} (µM) for Individual CYP Isoforms[a]					
	1A2	2B6	2C9	2C19	2D6	3A4
MB 1% USP	<0.05	2.4	1.0	<0.05	29	2.7
(b) (4)	<0.05	2.7	1.3	<0.05	30	4.0
Standard Inhibitor[b]	2.6	11	0.45	5.7	0.089	0.015

a= Average results of duplicate determinations.
b= Standard inhibitors: 1A2 – Furafylline, 2B6 – Tranylcypromine, 2C9 –Sulfaphenazole, 2C19 –Tranylcypromine, 2D6 – Quinidine, 3A4 – Ketoconazole
Source: Applicant's Report No.: BD00196

The applicant also evaluated CYP1A2 or CYP3A4 induction by (b) (4) or MB 1% USP using cryopreserved hepatocytes from three donors. Samples were incubated then analyzed by LC/MS/MS to determine the amount of CYP1A2 or CYP3A4 activity present (Table 5). The applicant concludes there is no induction; however these results are inconclusive given the poor performance of the active control and the poor hepatocyte viability at 100 µM. The induction results should not be reported in the labeling. Given the acute and limited nature of the proposed use of intravenous (b) (4) for the proposed indication, additional in vitro or vivo studies are not required at this time.

Table 5: In vitro fold induction (% of control) of CYP Isoforms 1A2 and 3A4 by MB 1% USP and Proviblue

Compound	Mean [range] % of control for Individual CYP Isoforms (n=3 donors)[a]		
	Concentration	1A2	3A4
MB 1% USP	1 µM	1.2 [1-1.3]	1.2 [0.9-1.4]
	10 µM	1.3 [1.2 -1.4]	1.6 [1.2-1.8]
	100 µM	0.03 [0-0.1]	0.4 [0.2-0.7]
(b) (4)	1 µM	1 [0.9-1.2]	1 [0.9-1.1]
	10 µM	0.8 [0.7-0.9]	1 [0.7-1.3]
	100 µM	0.1 [0-.2]	0.3 [0.1-0.4]
Standard Inducer[b]		1 [0.6-1.5]	1.7 [1.3-2.3]
Vehicle Control		1	1

a= Average results of duplicate determinations.
b= Standard inducers: 1A2 – Omeprazole 50uM, and 3A4 – Rifampicin 10uM
Source: Applicant's Report No.: BD00196

2.4.2.4 Is the drug a substrate and/or an inhibitor of P-glycoprotein transport processes?

The applicant did not provide any information or labeling recommendations related to the impact of hepatic impairment on MB or Azure B exposure following intravenous administration of (b) (4) or MB 1% USP. A comment should be sent to

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the applicant to explore potential transporter systems involved in MB handling in humans in vitro.

2.4.2.5 Are there other metabolic/transporter pathways that may be important?

The specific pathway that generates the metabolites Azure A and Azure B may be important to understand the impact of extrinsic factors on (b) (4) exposure. A comment should be sent to the applicant to consider exploring this issue in vitro.

2.4.2.6 Does the label specify co-administration of another drug (e.g., combination therapy in oncology) and, if so, has the interaction potential between these drugs been evaluated?

No.

2.4.2.7 What other co-medications are likely to be administered to the target patient population?

This is an agent designed to be dosed acutely in emergency situations. There are no specific medicines that would likely be administered in the target population. Patients will likely be on diverse treatments.

2.4.2.8 Are there any in vivo drug-drug interaction studies that indicate the exposure alone and/or exposure-response relationships are different when drugs are co-administered?

No.

2.4.2.9 Is there a known mechanistic basis for pharmacodynamic drug-drug interactions, if any?

In vitro studies and clinical case reports using MB 1% USP in the medical literature suggest that MB and its metabolite Azure B are inhibitors of MAO.[2 10 11] These reports suggest that tissue MB concentrations are sufficient to produce full MAO-A inhibition in humans at even the lowest doses (1–2 mg/ kg intravenously) reported in the medical literature for the treatment of methemoglobinemia.[11] Therefore, the labeling should include a statement in the drug interactions section that concurrent use with medicinal products that enhance serotonergic transmission including SSRIs (selective serotonin reuptake inhibitors), bupropion, buspirone, clomipramine, mirtazapine and venlafaxine should be avoided unless the benefit outweighs the risk and that if these drugs are used concurrently frequent monitoring for CNS effects id required .

2.4.2.10 Are there any unresolved questions related to metabolism, active metabolites, metabolic drug interactions, or protein binding?

Yes. The applicant should determine the metabolic and transporter based pathways for (b) (4) in vitro and as part of the mass balance trial discussed earlier in this review.

2.4.3 What issues related to dose, dosing regimens, or administrations are unresolved and represent significant omissions?

See Section 2.2.4.4

[10] Ramsay RR1, Dunford C, Gillman PK. Methylene blue and serotonin toxicity: inhibition of monoamine oxidase A (MAO A) confirms a theoretical prediction. *Br J Pharmacol.* 2007 Nov;152(6):946-51

[11] Gillman PK. CNS toxicity involving methylene blue: the exemplar for understanding and predicting drug interactions that precipitate serotonin toxicity. *J Psychopharmacol.* 2011 Mar;25(3):429-36.

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2.5 General Biopharmaceutics

2.5.1 Based on the biopharmaceutics classification system (BCS) principles, in what class is this drug and formulation? What solubility, permeability, and dissolution data support this classification?

Not applicable. This is an intravenous formulation.

2.5.2 What is the relative bioavailability/bioequivelance of the proposed to-be-marketed formulation to the reference listed product?

The applicant requests a waiver of relative bioavailability between (b) (4) and MB 1% USP. They cite self-evident bioavailability of an intravenous administration formulation and its belief that the difference in concentration (b) (4) between these products is inconsequential based on its interpretation of the results of its physical/chemical analysis as well as its in vitro protein binding and CYP inhibition trials. This issue was reviewed by ONDQA per memorandum of understanding with OCP and they informed the review team during the scheduled midcycle meeting for this application that a waiver cannot be granted because there is no listed drug to use as a reference for the waiver. The clinical pharmacology reviewer is also unable to link BA/BE to the literature because the PK information provided by the applicant for MB 1% USP is highly variable due to a combination of assay, formulation and sampling issues (see Section 2.2.5). Therefore, the applicant should conduct a relative BA/BE trail between (b) (4) and MB 1% USP to address this issue and potentially allow for the PK, efficacy, and safety information to be linked.

2.5.2.1 What are the safety or efficacy issues, if any, for BE studies that fail to meet the 90% CI using equivalence limits of 80-125%?

A BA/BE trial was not conducted.

2.5.2.2 If the formulations do not meet the standard criteria for bioequivalence, what clinical pharmacology and/or clinical safety and efficacy data support the approval of the to-be-marketed product?

Clinical pharmacology data do not support the approval of (b) (4) Approval of the to-be-marketed product can only be based on safety and efficacy endpoints in pediatric patients from the applicant's uncontrolled small retrospective trial using (b) (4) as well as other small uncontrolled retrospective trials and case reports from the medical literature that primarily used MB 1% USP. Due to the absence of PK/PD information in pediatric patients we defer to the Clinical reviewer regarding whether the observed safety and efficacy profile from the submitted trial and case study information justify approval. We caution against extrapolation of findings from MB 1% USP to (b) (4) due to the lack of relative BA/BE between these products.

2.5.3 What is the effect of food on the bioavailability (BA) of the drug from the dosage form? What dosing recommendation should be made, if any, regarding administration of the product in relation to meals or meal types?

Not applicable. This is an intravenous formulation.

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2.5.4 When would a fed BE study be appropriate and was one conducted?

Not applicable. Intravenous formulation

2.5.5 How do the dissolution conditions and specifications ensure in vivo performance and quality of the product?

Not applicable. Intravenous formulation

2.5.6 If different strength formulations are not bioequivalent based on standard criteria, what clinical safety and efficacy data support the approval of the various strengths of the to-be-marketed product?

Not applicable. One strength formulation is proposed.

2.5.7 If the NDA is for a modified release formulation of an approved immediate product without supportive safety and efficacy studies, what dosing regimen changes are necessary, if any, in the presence or absence of PK-PD relationship?

Not applicable. This is not a modified release formulation.

2.5.8 If unapproved products or altered approved products were used as active controls, how is BE to the approved product demonstrated? What is the basis for using either in vitro or in vivo data to evaluate BE?

See Sections 2.5.2 and 2.5.2.2

2.5.9 What other significant, unresolved issues related to in vitro dissolution or in vivo BA and BE need to be addressed?

See Section 2.5.2

2.6 Analytical Section

Not applicable. The applicant did not submit any trials for (b) (4) that identified and measured the active moieties in plasma or other mediums.

The applicant reports that in the "Luitpold 2013" trial using the American Reagent's MB 1% USP submitted with right of reference, the incurred sample reassay analysis from the original assay method revealed that the method was inadequate due to varying degrees of conversion between the reduced and oxidized forms of methylene blue and azure B. A new test procedure was developed employing (b) (4) (b) (4)

Due to multiple freeze/thaw cycles on the original tubes used on the rejected assays, the previously unused duplicate sample tubes were used for the reanalysis. Information regarding the handling and storage of the samples during the analysis and reanalysis for this study were not provided and the potential for this to effect the final PK parameter estimates cannot be ruled out.

3 DETAILED LABELING RECOMMENDATION

Not applicable for a CR action.

4 APPENDICES

4.1 Cited References

8 Pages of COPYRIGHT MATERIAL have been Withheld in Full immediately following this page

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This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

JOSEPH A GRILLO
09/03/2014

JULIE M BULLOCK
09/04/2014

NAM ATIQUR RAHMAN
09/04/2014
I agree with the recommendation.

Office of Clinical Pharmacology
New Drug Application Filing and Review Form

General Information About the Submission

Methylene blue is widely used in the U.S. to treat methemoglobinemia, vasoplegic syndrome, ifosfamide-induced encephalopathy, and cyanide poisoning. There is currently no methylene blue products approved by the FDA or listed in the Orange Book as a reference product. The Sponsor submitted a 505(b)(2) application using literature to support the proposed indication, but will not have a reference listed product for the application. (b) (4) . The current submission includes a PK trial in healthy volunteers that was conducted by the applicant, but it does not appear that the applicant's proposed (b) (4) product was used.

NDA/BLA Number:	204630	**SDN:**	009
Sponsor:	Provepharma SAS	**Date of Submission**	09/13/2013
Brand Name:	(b) (4)®	**Generic Name:**	Methylene Blue
Drug Class:	Antidote		
Dosage Form:	0.5 % Injectable (available in 10 mL ampules)		
Dosing Regimen:	(b) (4)		
Route of Administration:	Injection		
Indication:	Treatment of acquired methemoglobinemia (b) (4)		
OCP Division:	DCP5	**OND Division:**	DHP
OCP Reviewer:	Joseph Grillo, Pharm. D.		
OCP Team Leader:	Julie Bullock, Pharm. D.		
PM Reviewer:			
PM Team Leader:			
GG Reviewer:			
GG Team Leader:			
Priority Classification:	☒ Standard ☐ Priority	**PDUFA Due Date**	13-Sep-13
OCP Review Due Date:	13-Jul-14	**OND Division Due Date:**	13-Aug-13

Clinical Pharmacology and Biopharmaceutics Information

	"X" if included at filing	Number of studies submitted	Critical Comments
Table of Contents present and sufficient to locate reports, tables, data, etc.	☒		
Tabular Listing of All Human Studies	☐		
Human PK Summary	☐		
Labeling	☒		
Bioanalytical and Analytical Methods	☒		A summary of analytical methods was provided in the original 8/13/12.
I. Clinical Pharmacology			
Mass balance:	☐		

Isozyme characterization:	☐		
Blood/plasma ratio:	☐		
Plasma protein binding:	☒	1	Sponsor does not provide detailed study report (8/13/12 submission).
Pharmacokinetics (e.g., Phase I) -	☐		
Healthy Volunteers:			
single dose:	☒	1	Appears Sponsor used American Reagents product rather than [redacted] (b) (4) Dataset and bioanalytical report.
multiple dose:	☐		
Patients:			
single dose:	☐		
multiple dose:	☐		
Dose proportionality -			
fasting / non-fasting single dose:	☐		
fasting / non-fasting multiple dose:	☐		
Drug-drug interaction studies -			
In-vivo effects on primary drug:	☐		
In-vivo effects of primary drug:	☐		
Concomitant therapy:	☐		
In-vitro:	☒	1	Sponsor does not provide detailed study report (8/13/12 submission).
Subpopulation studies -			
ethnicity:	☐		
gender:	☐		
pediatrics:	☐		
geriatrics:	☐		
renal impairment:	☐		
hepatic impairment:	☐		
PD -			
Phase 2:	☐		
Phase 3:	☐		
PK/PD -			
Phase 1/2, proof of concept:	☐		
Phase 3 clinical trial:	☐		
Population Analyses -			
Data rich:	☐		
Data sparse:	☐		
QT evaluation:	☐		
II. Biopharmaceutics			
Absolute bioavailability:	☐		
Relative bioavailability -			
solution as reference:	☐		
alternate formulation as reference:	☐		
Bioequivalence studies -			
traditional design:	☐		
replicate design:	☐		
Food-drug interaction studies:	☐		
Bio-waiver request based on BCS	☐		
BCS class	☐		
Alcohol induced dose-dumping	☐		
III. Other CPB Studies			
Genotype/phenotype studies	☐		
Chronopharmacokinetics	☐		
Pediatric development plan	☐		
Literature References	☒		
Total Number of Studies		3	

On **initial** review of the NDA/BLA application for filing:

	Content Parameter	Yes	No	N/A	Comment
Criteria for Refusal to File (RTF)					
1	Has the applicant submitted bioequivalence data comparing to-be-marketed product(s) and those used in the pivotal clinical trials?			x	
2	Has the applicant provided metabolism and drug-drug interaction information?	x			In-vitro CYP DDI data (not detailed)
3	Has the sponsor submitted bioavailability data satisfying the CFR requirements?			x	
4	Did the sponsor submit data to allow the evaluation of the validity of the analytical assay?		x		Complete validation report not provided
5	Has a rationale for dose selection been submitted?			x	
6	Is the clinical pharmacology and biopharmaceutics section of the NDA organized, indexed and paginated in a manner to allow substantive review to begin?	x			
7	Is the clinical pharmacology and biopharmaceutics section of the NDA legible so that a substantive review can begin?	x			
8	Is the electronic submission searchable, does it have appropriate hyperlinks and do the hyperlinks work?	x			
Criteria for Assessing Quality of an NDA (Preliminary Assessment of Quality)					
	Data				
9	Are the data sets, as requested during pre-submission discussions, submitted in the appropriate format (e.g., CDISC)?		x		Datasets from PK trial not submitted
10	If applicable, are the pharmacogenomic data sets submitted in the appropriate format?			x	
	Studies and Analyses				
11	Is the appropriate pharmacokinetic information submitted?		x		Applicant's PK trial does not appear to use (b) (4) Conflicting results from the published trials submitted
12	Has the applicant made an appropriate attempt to determine reasonable dose individualization strategies for this product (i.e., appropriately designed and analyzed dose-ranging or pivotal studies)?			x	
13	Are the appropriate exposure-response (for desired and undesired effects) analyses conducted and submitted as described in the Exposure-Response guidance?			x	
14	Is there an adequate attempt by the applicant to use exposure-response relationships in order to assess the need for dose adjustments for intrinsic/extrinsic factors that might affect the pharmacokinetic or pharmacodynamics?			x	
15	Are the pediatric exclusivity studies adequately designed to demonstrate effectiveness, if the drug is indeed effective?			x	
16	Did the applicant submit all the pediatric exclusivity data, as described in the WR?			x	

17	Is there adequate information on the pharmacokinetics and exposure-response in the clinical pharmacology section of the label?		x		Conflicting PK information in the published and applicant trials. No E-R provided
	General				
18	Are the clinical pharmacology and biopharmaceutics studies of appropriate design and breadth of investigation to meet basic requirements for approvability of this product?		x		PK trial does not appear to use (b) (4)
19	Was the translation (of study reports or other study information) from another language needed and provided in this submission?			x	

Is the Clinical Pharmacology Section of the Application Fileable?

☒ **Yes**

☐ **No**

If the NDA/BLA is not fileable from the clinical pharmacology perspective, state the reasons and provide comments to be sent to the Applicant:

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

The following issues will need to be addressed:

1. (b) (4)

2. **Regarding the trial AA98923: "A One-Period, Single-Dose, Safety, Tolerability, and Pharmacokinetic Study of Methylene Blue Injection USP Following a 1 mg/kg Intravenous Dose in Healthy Adult Volunteers provide the following within 10 business days**

   a. **It appears that the methylene blue product used in this trial was not your (b) (4) formulation. Please confirm this and if true please explain how this trial supports the approvability of (b) (4)**

   b. **We note that the final study document reports PK parameters as arithmetic mean (SD) only and the linear time concentrations plot provided is a mean plots without variability. Update the final study report results such that summary statistics for the PK parameters are reported in terms of arithmetic mean, SD, CV, geometric mean, geometric CV, median, range. Time concentration plots should be plotted using both linear and semilog scales and include appropriate variability.**

   c. **Provide a PK Analytical report that includes the raw concentration data by subject, calculated PK parameters by subject, the model reports from your NCA analysis (such that your analysis may be reproduced by the Agency) and individual concentration time plots both methylene blue and its metabolite using linear and semilog scales.**

   d. **Provide complete electronic datasets for this trial in SAS transport file format (*.xpt). Provide all concentration-time and derived PK parameter datasets. In addition, datasets for all other domains related to safety (e.g., ADR's),**

demographics, non-PK laboratory values, concomitant drug use should be included.

e. Provide a complete bioanalytical report to justify the validation of the assay used to quantify methylene blue and the Azure B metabolite

3. We note that you have not addressed the comments we provided to you previously regarding conflicting PK results in the literature, (b) (4) further in vivo evaluation of CYP inhibition, evaluation of the potential for CYP induction, evaluation of the potential as a substrate, inhibitor, or inducer or transporter systems, and the potential QT/QTc interval prolongation. These will not be accepted for review as part of this submission after the filing date.

Addendum:

The applicant provided a response on 11/19/13 and additional revised information on 11/25/13. Overall the sponsor states that 1) the requested in vitro study reports can be found in the original NDA submission that was RTF, Trial AA98923 is to support a biowaiver, additional information for trial AA98923 were provided. There is no additional information regarding comment #3 beyond the applicant's response to deficiencies document included in the submission. No additional action required at this time.

Signatures:

Joseph Grillo, Pharm.D.
Reviewer
Division of Clinical Pharmacology 5

Julie M. Bullock, Pharm.D.
Team Leader
Division of Clinical Pharmacology 5

Clinical Pharmacology - NDA Filing Memo

NDA:	**204630/009 NDA Resubmission following RTF**	**IND: 113,942**
Compound:	**(b) (4)® (Methylene blue) 0.5% solution for injection**	
Sponsor:	**Provepharm SAS**	
Submission Date:	**September 13, 2013**	
Reviewer:	**Joseph Grillo, Pharm.D.**	

The sponsor has submitted a 505 (b)(2) application for (b) (4) (methylene blue) 0.5% injection primarily using historical literature data to support the proposed indication, but does not have a reference listed drug for the application. The current submission includes a PK trial in healthy volunteers that was conducted by the applicant, but it does not appear that the applicant's proposed (b) (4) product was used.

(b) (4) Injection, 0.5% is an antidote proposed for the treatment of acquired methemoglobinemia (b) (4) treatment of acquired methemoglobinemia. The proposed recommended dose (based on literature) in adults (b) (4):

(b) (4)

(b) (4)

The sponsor also provided the results of a single dose, one period, safety and PK study of MB 1%, 1 mg/kg, IV (American Reagent) in healthy volunteers in addition to several PK trials in the literature to support its application(See table below).

Trial	Cmax (normalized to 100 mg) µg/mL	AUC (normalized to 100 mg) µg/mL*hr
Peter et al	2.56 (2.56)	N/A
Repici et al	2.07 (2.07)	13.47 (13.47)
Walter-Sack et al	0.75 (1.50)	7.60 (15.20)
CSR No AA98923	0.49 (1.00)	3.07 (6.07)

None of these trials appear to have used (b) (4) In addition, A relative BA/BE trial was not provided by the applicant. As the table above suggests, there continues to be significant variability in the reported exposure and PK of methylene blue in both the literature and applicants report that remains unresolved. This poses a significant review issue for the applicability of these data to substantiate PK claims in the (b) (4) application from a clinical pharmacology perspective.

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

JOSEPH A GRILLO
12/18/2013

JULIE M BULLOCK
12/30/2013